EFFECT OF STORAGE ATMOSPHERE ON SEED VIABILITY AND VIGOUR IN *PINUS RADIATA*, D. DON

by

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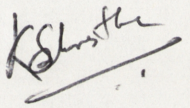
STATEMENT OF ORIGINALITY

Except where specific acknowledgement is given, the research work reported in this thesis is entirely that of the author.



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SUMMARY

Plantation programmes to establish new forests are expanding rapidly especially in the developing countries of the tropics. As such, seed is the first requirement in the silvicultural processes for developing such forests, whether with direct seeding or by planting stock. For continuous production of planting stock or for direct seeding, sufficient quantities of seed need to be maintained because forest trees exhibit crop periodicity. Therefore, storage of seed is a practical necessity for artificial regeneration works.

Seed storage has been a problem in the developing countries because storage methods which rely on the control of temperature by refrigeration and the maintenance of seed moisture content by dehumidification equipments demand capital investment and high technology. These are lacking in the developing countries. In such countries, relatively simple methods of seed storage are needed to maintain the physiological quality of the seed.

Relatively simple methods of seed storage by controlling the storage atmosphere were examined on seed of *Pinus radiata*. Reduction in seed deterioration was achieved by replacing or limiting the oxygen supply and by maintaining the seed moisture content in sealed laminated plastic bags. Of the four storage atmospheres used, an atmosphere of air was less effective than vacuum, carbon dioxide and nitrogen, in that order in maintaining the viability and

vigour of the seeds. Storage in an atmosphere of nitrogen was most effective in prolonging the viability and vigour at all the storage temperatures employed ranging from 5°C to 35°C. Presence of oxygen in the storage atmosphere has deleterious effects on seed viability and vigour.

In developing countries, such relatively simple and cheap storage methods can be employed to improve storage condition. The methods employed here on *P. radiata* should be applicable to other orthodox seeds used in the tropics and subtropics. Depending upon the time period of storage, a particular storage atmosphere can be employed. Nitrogen storage would be advantageous for long term storage, carbon dioxide for medium term storage. In the field areas, where short term storage would be sufficient, storage of low moisture content seed in sealed container in air or in a vacuum would be the simplest and cheapest methods to maintain viability and vigour of seed.

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CHAPTER 1

INTRODUCTION

Natural forests around the world are being cleared for agricultural development at an alarming rate. In addition to this deforestation large areas of forest are being logged for timber and are frequently being degraded due to overexploitation (mainly for fuelwood), overgrazing and repeated burning. The establishment of new forests on cleared or degraded forest land to supply the wood requirements of expanding populations is proceeding rapidly, especially in tropical regions. A recent survey by the Food and Agriculture Organisation of the United Nations showed that an existing plantation area of 11.5 million ha in 76 tropical countries is increasing at an annual rate of 1.1 million ha (Lanly 1982).

Man-made forests are created to fulfil a variety of objectives, ranging from the production of raw materials for paper-making and wood-based industries to fuelwood and simple products for building in village situations. They can be a most efficient and economical way of providing raw material for forest-based industries and for many other non-industrial uses.

In the tropical regions of Asia the natural regeneration of some preferred species is too slow and too erratic to be relied upon as the sole means of increasing wood production to meet national requirements. In some developing countries, such as the

Philippines, Thailand and Nepal, overexploitation and shifting cultivation have resulted in considerable depletion of the natural forests. For these reasons increased reliance is being placed on artificial regeneration methods - either the raising of man-made forests or, less frequently, the enrichment of the natural forests.

In Africa, as in the Asian region, the indigenous hardwood forest areas are large and important both economically and socially. However, because of their mixed composition, slow growth rate and special timber properties, they cannot provide the bulk of the growing need for industrial wood. There is, therefore, a shift in emphasis from natural regeneration methods towards man-made forests in most African countries (Britwum 1973).

In recent years, there has been an increasing recognition in the developing countries of the role of trees and forests to alleviate energy problems and to conserve the environment. Increasing emphasis is therefore being laid on afforestation or reforestation with fast-growing trees for the production of fuelwood and protection of watershed areas.

Seed is of fundamental importance for afforestation or reforestation purposes, whether with indigenous or exotic species. Large quantities of seeds are required annually throughout the world for artificial regeneration. However, many tree species exhibit periodicity in their seed crops with heavy crops often being several years apart. For such species the forester needs to obtain seeds

during a good seed year and to store it for use in the intervening years of poor supply. This ensures a continuous supply of seed for annual production of planting stock or for direct seeding.

The success of any planting programme depends upon the supply of well-adapted vigorously growing seedlings. The seedling performance depends very much on the quality of the seed used. Seed quality is a broad term which embraces seedlot characteristics such as genetic constitution, germination percentage, vigour and purity. The seed storage method used must maintain a high proportion of seeds capable of vigorous germination. Seeds which germinate very weakly may be considered no better than dead seeds. The use of good quality seed enhances nursery efficiency and reduces costs.

Seeds can lose viability through the protracted deterioration processes associated with aging and storage conditions. The effects of deterioration manifest themselves in a variety of ways, the most readily appreciated being a decline in germination capacity. However, often a change in vigour of the germinating seedlings will occur before any change in germination capacity of the seedlot is evident. The fundamental reason for storage is to preserve or maintain the physiological quality of seed by minimising this rate of deterioration. Being a living unit, seed must deteriorate in storage. The exact reason for seed deterioration in storage is not known (Roberts 1972c) though many theories, such as accumulation of mutagenic substances, depletion of stored reserves and nucleic damage, have been proposed.

Seed storage has been practised for several thousand years in agriculture but in forestry for only about 100 years (Simak 1980). Various methods have been employed for medium- or long-term storage but most involve the control of temperature or seed moisture content. Storage facilities, including refrigerated rooms and dehumidified areas, have been developed to achieve this control and are widely used in countries with a more advanced technology.

In most Asian and African countries, as in many other developing countries, the problem of seed storage had been neglected for a long time due to lack of proper storage facilities. Often quite primitive methods are used, such as storage in the open or underground burial in sand (Hasan 1973). With increasing emphasis given to artificial regeneration, seed storage has received more attention. Reliance is now being placed on more sophisticated techniques of storage developed elsewhere. The use of refrigerated storage requires capital investment, proper maintenance and a reliable source of electricity. Unfortunately, these are not readily available in many developing countries. In such countries relatively simple methods of seed storage are needed which do not rely on high-cost installations or the availability of trained maintenance staff.

In this study relatively simple methods of controlling the seed storage atmosphere are examined. The purpose was to determine whether the methods used would be successful in maintaining viability and vigour of tree seed. Storage experiments have been carried out

with some agricultural seeds to examine the effects of low oxygen pressure or oxygen-free atmosphere during storage on viability and vigour. The results have been variable (Bewley and Black 1982). There has been very little investigation into this aspect of seed storage in forest tree species.

The development of laminated plastic sheeting, almost completely impermeable to moisture and gases, provided the opportunity to provide a cheap and effective sealed storage bag. Such sealed containers have been very effective for the storage of rice food grains (Mitsuda *et al.* 1973) and frozen beef. This material could be readily adopted for the sealed storage of forest tree seed.

The species selected for this study was *Pinus radiata* D. Don, radiata pine. Species of this genus are planted extensively in many developing countries and the results obtained for *P. radiata* may be directly applicable to other pine species. The reasons for choosing this species were, first, an adequate supply of seeds were available and, second, no studies of controlled atmosphere storage have been made on *P. radiata* which is the most widely planted species in Australia.

Aims of the study

The aims of the study reported in this thesis were;

(a) to compare the effects of sealed storage atmospheres of air, vacuum, carbon dioxide and nitrogen gas on the germinability

and rate of germination of seeds stored at low moisture content, and

(b) to evaluate the effects of these storage conditions on the seed vigour (as measured by seedling growth) after various periods of storage up to 1 year.

Ideally, a study of this nature should have been pursued for a longer period of time but as it was a graduate research study it had to be of comparatively short duration. However, a range of higher temperatures were employed to hasten the physiological ageing process during storage and to accelerate the rate of physiological deterioration. For even more accelerated ageing, higher temperatures combined with higher air humidity could have been employed. However, this would have involved much more complex procedures which were not warranted.

Chapter II contains a general review of the literature concerning this study. Chapter III is concerned with the germination experiments following storage treatments of the seed. Experiments on growth energy of seedlings following storage treatments of the seed are included in Chapter IV. Chapter V is concerned with a general discussion and conclusions.

CHAPTER 2

LITERATURE REVIEW

2.1 SEED STRUCTURE

A seed is essentially simple in structure. It is a matured ovule containing an embryo and, in many cases, an endosperm, all enclosed in a seed coat or testa. The embryo is a miniature plant made up of a radicle, a plumule or epicotyl, one or more cotyledons and a hypocotyl connecting plumule and radicle.

The embryo is usually derived from the fusion of nuclei of male and female gametes i.e. it is the result of the fertilisation of the egg cell in the embryo sac by one of the male nuclei from the pollen tube. The testa develops from the integument(s) of the ovule. The endosperm in conifers is female gametophyte tissue of 3n nature but devoid of male contribution.

The testa is a protective structure. The endosperm is a food-storage organ but in some species it degenerates, remaining as a rudimentary tissue, and the cotyledons take over its storage function. The seed contains a variety of nutrients, principally carbohydrates, lipids and proteins, which are used for the purpose of respiration during storage and mobilised rapidly for growth at the time of germination (Esau 1977).

A seed is a closed system containing all the stored energy and reserve nutrients necessary for initial germination, including a metabolic component necessary to utilise the reserves, and information essential to direct mobilisation of them (Pollock 1972). A seed has been described by Roberts (1972a) as 'an embryonic plant in a resting stage'.

2.2 SEED VIABILITY AND LONGEVITY

A viable seed is one which is alive and potentially capable of germination (Roberts and Ellis 1982). Viable seed can germinate under favourable conditions providing any dormancy that may be present is removed. Thus a non-viable seed is one which will not germinate when given near optimum conditions, such as adequate temperature, a supply of water and some oxygen (Roberts 1972a). However, seed viability can be increased, especially in older, stored seed, by allowing them to imbibe water slowly and thus apparently restore membrane integrity. This allows seed to germinate which otherwise could not, as with seed prevented by dormancy factors from germinating when placed in otherwise near optimum conditions for germination.

A seed does not change abruptly from a viable into a non-viable state when it ages but passes through various stages of deterioration (Harrington 1972; Delouche and Baskin 1973; Bewley and Black 1982). The symptoms of deterioration are:

(a) change in seed colour,

(b) delay in radicle emergence and seedling growth,

(c) lower tolerance to adverse storage conditions,

(d) decline in tolerance to sub-optimal conditions during germination,

(e) greater susceptibility to attacks by micro-organisms at its environmental extremes,

(f) abnormality in germination, and

(g) inability to germinate.

As deterioration in a seedlot is on an individual basis, the ageing of a seedlot is not uniform but is a function of the past history of each individual seed in the lot (Harrington 1972). A seedlot, therefore, may consist of seeds varying in degree of deterioration from a relatively non-deteriorated to a non-germinable condition although they may be genetically and physically similar (Delouche and Baskin 1973).

All seeds in a lot do not have the same life span; they age and die at different rates. Differential rates of deterioration result in 3 distinct phases during a seedlot's storage life:

(a) a period of high viability in which relatively little deterioration occurs,

(b) a relatively short period in which germination declines sharply to lower level, and

(c) a relatively slower rate of decline until all seeds die.

Roberts and Ellis (1982) noted that for orthodox seeds under any constant storage condition, the frequency distribution of seed deaths in time is normal. The seed survival curve is a cumulative normal

distribution of negative slope when percentage viability is plotted against time in storage.

The fundamental causes of deterioration and loss of viability of seeds in storage are still not understood well (Pitel 1980). Many theories on cause of loss of viability of seeds have been put forward. Roberts (1972c), however, had divided these theories into two groups:

(a) Extrinsic - in which the activities which cause death of the seed originate from outside the seed.

(b) Intrinsic - in which death is a result of events occurring within the seed.

Extrinsic theories include the activity of fungi and effect of background ionising radiation on the loss of viability of seed. Roberts (1979) while reviewing the causes of loss of viability, however, noted that although extrinsic agents can contribute to loss of viability, they are seldom a major or sole cause. Intrinsic theories encompass hypotheses, such as accumulation of toxic substances or mutagens, depletion of essential reserve materials, loss of integrity of cell membrane and nuclear damage. Roberts and Ellis (1982), however, noted that many sub-cellular systems are affected during seed deterioration and it is by no means clear which are the most significant in reducing vigour or what is the immediate cause of death.

The period for which the seeds remain viable is extremely variable and depends both on storage conditions and the type of seed (Mayer and Poljakoff-Mayber 1975). The life span of seed, or longevity, varies greatly among species (Kozlowski 1971). Crocker and Barton (1957) noted that some kinds of seeds are inherently short-lived while others are long-lived. Ewart (1908) divided seeds into three biological classes according to their life span under optimal (undefined) conditions. They are:

(a) microbiotic - seeds having a life span of up to 3 years,

(b) mesobiotic - seeds having a life span from 3 to 15 years,

(c) macrobiotic - seeds having a life span above 15 years.

Baldwin (1942) maintained that most forest tree species are mesobiotic and that macrobiotic species are rare except for the Leguminosae and Rosaceae families.[1] Microbiotic behaviour is reported for many common temperate genera, such as *Juglans*, *Salix* and *Quercus* (Holmes and Buszewicz 1958). Harrington (1972) while commenting on these three biological classes of seeds, however, pointed out that the role of environmental or physiological factors was not considered when the longevity of given sample of seeds was determined. Bewley and Black (1982) argued that this classification of longevity is not particularly satisfactory because for many seeds the most favourable storage environment has not been determined and added that the categories have little meaning unless this information is obtained. As such when the storage condition for any given seed is improved, it may change from the micro- to the meso-biotic class. Harrington (1972) classifed seeds into 2 classes:

[1] There is now ample evidence to show that Baldwin (1942) based this on limited knowledge of seed longevity as many conifers of the north temperate forests produce macrobiotic seeds, rarely do they produce mesobiotic seeds.

1. short-lived seeds - having longevity from a few days to a few years.
2. long-lived seeds - having longevity of more than 10 years.

However, he also admitted that the record of seed longevity exists for only a few of the many species of plants, and often for seed stored under less than ideal conditions.

Holmes and Buszewicz (1958) and Harrington (1972) noted that seeds having a hard and impermeable seed coat usually have a long life span. This structure seems to effectively seal the seeds against the environment, minimising moisture exchange and loss of stored reserves through respiration activity. In contrast, seeds with thin coats often have a life expectancy of only a few weeks or months in open air (Hatano and Asakawa 1964).

2.3 FACTORS AFFECTING VIABILITY OF SEED IN STORAGE

The viability of seeds in storage is influenced by:

1. the physiological condition of the seed and the treatment which the seed has received prior to storage, and
2. the environmental factors during storage

2.3.1 Pre-storage factors

Delouche *et al.* (1973) noted that the condition to which seeds are exposed prior to storage and the trauma sustained during

pre-storage operations determines their response in a specific storage condition. Owen (1956) reported that seeds of high initial viability are much more resistant to adverse storage conditions than those of poor viability. Seed of low viability tends to deteriorate more rapidly under any given condition. The pre-storage factors which are of most importance in affecting seeds are;

1. maturity,
2. environmental stress during seed formation, and
3. injury to the seed.

Maturity: Baldwin (1934) reported that seeds lacking endogenous dormancy attain their optimum germination capacity when allowed to ripen fully before collection. Baldwin (1942) further noted that only thoroughly ripened seeds can retain their viability for long, since immature seeds not only are lacking in embryo development but may not, yet, have accumulated their full stock of reserve food or the reserves have not been changed into stable compounds which can withstand drying. Austin (1972) stated that until the attainment of full maturity full germination capacity and viability are not attained. Unripe seed may have an immature embryo (Holmes and Buszewicz 1958). Immature seeds when dried cannot be stored for long periods before they die (Harrington 1972). For example, Holmes and Buszewicz (1958) cite the work of Rohmeder (1942) who showed that unripe seeds of *Ulmus* species were more affected by changes in storage temperature than mature seeds. Overmature seeds have shorter longevity than mature seeds (Harrington 1972), that is seeds which have passed the point of full maturity and have begun to senesce.

Stress: Variation in the environment of seeds around the time of completion of maturation and of harvesting can result in different potential viability period (Bewley and Black 1982). Environmental stress resulting from water deficits, high or low temperatures and mineral deficiency to the growing mother plant during seed development can reduce the storage life of the mature seeds produced. Seeds from lettuce and carrot grown under conditions of severe deficiency in nitrogen, potassium or calcium declined more rapidly in germination capacity during 8 years of storage than did seeds from plants given a balanced nutrition (Harrington 1972). However, Austin (1972) states that unless the mineral deficiency is severe it has relatively minor effects.

Temperature during seed ripening can influence the subsequent performance of seeds in various ways. Heat of high intensity may cause damage to the seed that may lead to a rapid loss of viability in storage. On the other hand, freezing injury as the seed matures may adversely affect the keeping quality of the seed (Austin 1972). Rossman (1949) noted that seed of corn plants hit by early frost during seed ripening had a shortened storage life.

Lack of water in the soil or excessive transpiration when the seed is in the rapidly developing stage may damage the development of the embryo. Such seeds when placed in storage may deteriorate more rapidly than other seeds which are more mature or had not yet reached that stage of maturity (Harrington 1972). Austin (1972), however, contended that unless the environmental

variation is extreme, it has very little effect on the viability of seeds of most species.

Injury: Physical damage sustained to seeds in general handling, extraction, dewinging and cleaning process impairs viability. Allen (1957) reported that an impact just to crack the seed coat could kill the seed of *Pseudotsuga* species. Baldwin (1942) noted that injury from dewinging machine may not be apparent from inspection of the seed but may appear upon germination through abnormally wide splitting of the seed coat upon emergence of the radicle. Eliason and Heit (1940) found the same for *Pinus resinosa*. Such seedlings are less vigorous and more susceptible to injury. Chin (1976) noted that seedlings from mechanically damaged seeds were very weak and stunted compared with the vigorous seedling from the undamaged seeds of soya bean cv. 'Harosoy'. Barton (1961) maintained that mechanical injury to seeds by threshing usually contributes to immediate reduction in germination capacity and to an accelerated loss of viability in storage.

The nature of mechanical damage varies widely. The number and intensity of impacts to which seeds are subjected may greatly influence the extent and seriousness of mechanical damage. The most intensive injury to seeds reduce their viability immediately while small injury often does not cause immediate loss in viability but become increasingly critical as ageing occurs (Moore 1972). He had further shown that very moist seeds tend to bruise readily during impact whereas drier seeds tend to fracture involving physical

breakage or internal cracking. High moisture content associated with bruising may greatly hasten the rate of deterioration. Bewley and Black (1982) noted that injured or deeply bruised areas may serve as centres for infection and result in accelerated ageing. They may also promote weakening and early death of surrounding normal tissues. Injuries close to vital parts of the embryonic axis may bring about a more rapid loss of viability during storage than injuries located elsewhere. Tyzkiewicz (1952) reported that seed damaged by mechanical means respire more intensively than normal under all conditions and are useless for long term storage (cited by Holmes and Buszewicz 1958). Prokobboon (1982) states that soya bean seeds damaged by threshing started to deteriorate and lose viability faster than undamaged seeds. He also noted that seed threshed by hand have a higher germination percentage and a lower percentage of abnormal seedlings than beaten and machine threshed seeds.

2.3.2 Factors during storage

Owen (1956) noted that the life span of seed of any species, provided it is sound, thoroughly matured and of high initial viability, is largely dependent on environment and added that the principal factors influencing the viability of such seed in storage are moisture content of seed and temperature. Bass (1979) reported that seed moisture content and storage temperature are very important factors in seed longevity, with seed moisture content usually regarded as the most influential of the two. Bewley and Black (1982) reported that the factors which most influence longevity of seeds in storage are;

(a) moisture content,

(b) temperature, and

(c) oxygen pressure.

In other words, the environmental factors have a decisive effect on the life span of any seed i.e. whether the seed will remain viable for the longest period possible or whether it loses its viability at some earlier stage (Mayer and Poljakoff-Mayber 1975).

Moisture Content: Seeds are hygroscopic. They absorb moisture from the atmosphere or lose moisture to it until the seed moisture and atmospheric moisture reach equilibrium. The rate of gain or loss of moisture will depend on the moisture gradient between the seed and the atmosphere, so that the rate of moisture loss of drying seed decreases as moisture content approaches that of dry air. Thus different kinds of seed attain specific, or characteristic, moisture contents when exposed to different levels of atmospheric relative humidity. This is known as the equilibrium moisture content. This is because the molecules in the seed vary in the amount of water they absorb, for example, proteins adsorb more water than starch and cellulose, but lipids adsorb no water (Harrington 1973). So increase or decrease in relative humidity of the air directly affects the moisture content of the seeds if the atmospheric air is in contact with the seeds in storage. The maintenance of viability in seeds is closely associated with their moisture content. For most species a seed moisture content of 5-6% maximises the storage life (Harrington 1973). Seeds that can be dried to low moisture content,

5% or less, without damage, and under appropriate conditions stored for long periods are termed 'orthodox' (Roberts 1973). Seeds to which the converse applies are known as 'recalcitrant'. The species of many commercial tree genera, such as *Betula, Eucalyptus, Pinus, Populus* and *Tectona* are orthodox but recalcitrant genera include *Acer, Dipterocarpus, Hopea, Juglans* and *Quercus* (Wakeley 1954; Tang and Tamari 1973; and Bonner 1978).

When seeds are dried below 5% moisture content the speed of deterioration may be faster than if maintained at a moisture content 1-2% higher due to damage from seed lipid autoxidation. On the other hand, a moisture content above 12% predisposes seeds to rapid deterioration due to fungal invasion and heating due to the respiration of the seed and micro-organisms. At seed moisture content over 40% non-dormant seed may germinate (Bewley and Black 1982).

Some light-sensitive crop seeds retain full germination capacity when in a fully imbibed condition at high temperatures but are prevented from germinating by storage in the dark (Villiers 1973). The apparently contradictory finding that while an increase in moisture content reduces the longevity of seeds in 'air-dry' storage, fully imbibed seed have a greatly extended life span is explained by Villiers and Edgecumbe (1975) on the basis that activation of sub-cellular repair and turnover mechanisms in hydrated seeds prevents the accumulation of internal damage during storage. Roberts (1981) supports the repair and turnover mechanism hypothesis

of Villiers but suggests that technical difficulties are likely to prevent its application as a practical technique for the storage of orthodox species.

When seeds with a moisture content above 14% are stored at sub-freezing temperature, the free water contained in their tissues freezes forming ice crystals which damage the cells and reduce viability (Harrington 1973). The moisture content at which such freezing damage occurs varies with temperature, at -20°C it may be in the region of 15% or a little above (Roberts 1972b). Seeds with a moisture content below 14% should retain their viability for a long period when stored at sub-freezing temperatures (Bewley and Black 1982).

Seed are stored commonly at temperatures just above freezing (1-5°C). At these low temperatures the relative humidity is normally high and the seeds gain moisture unless the humidity of the storage area is artificially reduced or the seeds are kept in moisture-proof containers. Storage at a constant humidity is desirable as Owen (1956) reported fluctuation in seed moisture content can accelerate their rate of deterioration.

Temperature: Temperature is the other main environmental factor, besides moisture content and relative humidity, affecting the viability of seed in storage. At high temperatures seed deteriorate more rapidly. How this happens is not known but possibly enzymic reactions are involved (Harrington 1972). Also, at high

temperatures, the activity of micro-organisms become conducive to seed deterioration. Christensen (1973) noted that the optimum temperature for growth of most storage fungi is about 30°-33°C and the maximum temperature is about 50-55°C.

At lower temperatures the activity of fungi and insects are reduced or stopped (Harrington 1973). In general for orthodox seeds, the lower the temperature, the longer the period of viability of seeds (Duffus and Slaughter 1980; Bewley and Black 1982). The longevity of orthodox seeds can be improved by storing them at sub-zero temperatures. The International Board for Plant Genetic Resources (1982) recommends orthodox seeds to be kept at -20°C with a moisture content of about 5% for long term storage. Lower temperatures could be used but are impractical on the basis of cost (Roberts 1979). Recalcitrant seeds, however, cannot be stored at sub-zero temperatures without damage and loss of viability.

Temperature and moisture content may interact in their effect on the metabolic rate of seed. Wakeley (1954) emphasized that maintaining a favourable combination of temperature and seed moisture content is far more important to successful storage than is choice of temperature or initial seed moisture alone. Delouche *et al.* (1973) maintained that the temperature and seed moisture content reinforce and compensate each other in their effect on longevity of seed. For optimum storage life orthodox seeds are stored at sub-zero temperatures (e.g. -20°C) and with a moisture content of about 5% (IBPGR 1982).

Oxygen Pressure: Dry seeds have an internal atmosphere containing oxygen in the cavities between seed structure and in the intercellular spaces. The permeability of the dry seed coat may be sufficient to allow the internal atmosphere to equilibrate with the air outside which contains 21% oxygen and 0.03% carbon dioxide. If the composition of the external atmosphere changes so too does the internal atmosphere of the seed. The composition of the storage atmosphere then affects the viability of the seed. Peterson *et al.* (1956) showed the deleterious effects of oxygen on the viability of wheat seeds, having 18% moisture content stored at 30°C in a mixture of nitrogen and oxygen. Seeds aspirated with 0.2% oxygen were 86% viable after 16 days, when the oxygen content was raised to 2.4% the viability fell to 38% and when aspirated with air the seed retained only 7% viability. Harrison (1966) found that increased availability of oxygen caused an increase in the rate of seed deterioration. In experiments he carried out on 10 varieties of lettuce seeds stored for 3 years at 18°C and 6% moisture content he found that the seeds which were sealed in oxygen had mean germination of 8% at the end of the experiment while seed stored unsealed had 48% germination. Roberts and Abdalla (1968) also reported a deleterious effect of oxygen on the viability of barley, beans and peas at 25°C and at 18% and 25% moisture content. The major effect was produced by increasing the oxygen level from 0% (nominal) to 21% at atmospheric pressure but a further increase to 100% had comparatively little effect. The reduction in viability was more pronounced at high moisture content, an observation also supported by Harrington

(1972). Roberts (1972b, 1973) generalized that an increase in the partial pressure of oxygen can lead to a decreased period of viability and these effects are greatest at higher temperatures and moisture contents. Bewley and Black (1982) concluded that reduction of oxygen concentration increase the viability of seeds whether this be achieved by vacuum or by raising the partial pressure of carbon dioxide, nitrogen, argon or helium.

Research into the effect of storing seeds in different atmospheres has been undertaken for many years. In 1914 Kidd prolongd the life span of *Hevea brasiliensis* by sealing the seeds in an atmosphere containing 40-45% carbon dioxide and storage in nitrogen gas was even more effective (Mayer and Poljakoff-Mayber 1975). The use of artificial atmosphere, either to reduce, or completely to exclude oxygen, did not noticeably affect the rate of deterioration of viability of seed of Chewing's fescue grass (Gane 1948) and on seeds of *Quercus* and *Fagus* species (Holmes and Buszewicz 1958) but others e.g. Harrington (1972), stated that high carbon dioxide, nitrogen or a vacuum may retard deterioration of seeds in storage. Harrison and McLeish (1954) showed that lettuce and onion seeds sealed in an atmosphere of carbon dioxide for 3 years retained a much higher germination capacity than seeds stored unsealed or in air sealed containers. Peterson *et al.* (1956) showed that increase in carbon dioxide concentration from 18-50% in the gas mixture containing 21% oxygen had beneficial effect on viability of wheat seeds stored for 20 days at 30°C and 18% moisture content and added that further increases resulted in higher viability. Glass *et al.*

(1959) reported that wheat seeds having 13-18% moisture content when stored at 30°C the atmosphere of nitrogen did not prevent, or noticeably delay, a loss in viability. In trials at 20°C, storage in nitrogen improved the storage life of the seeds. Harrison (1966) in a series of sealed storage experiments on lettuce seeds showed the advantage of storing in oxygen-free atmosphere, such as nitrogen, carbon dioxide or in a vacuum. Seeds stored for 3 years, at 18°C and 6% moisture content, in nitrogen and carbon dioxide gave a mean germination of 78% and in vacuum 77% while those sealed in air gave 57%. Harrison further demonstrated the advantage of sealing seed in nitrogen in that even at 50°C lettuce seeds with 6% moisture content stored for 6 weeks gave 80% germination while those stored in carbon dioxide and air failed to germinate after 5 weeks. Roberts and Abdalla (1968) found that, in an atmosphere of nitrogen, viability was considerably higher than seeds placed in replenished air or oxygen for a similar time period but not much greater than those hermetically sealed in air. They showed that pea seeds with 18% moisture content stored at 25°C in constant gaseous conditions of nitrogen took 91 days to fall to 50% viability (the half viability period) whereas those in replenished air and oxygen took 56 days as compared to 82 days by those hermetically sealed in air. This was explained by a linear decrease in oxygen from 21% to 1.4% and increase in carbon dioxide from 0.03% to 12% in air tight storage in 11.3 weeks - the time taken to reach 50% viability. Shejbal (1980) reported that grain quality, such as germination capacity and energy was maintained significantly longer in technical nitrogen (residual oxygen 0.2 - 0.5%) than in air at all temperatures from 10° to 30°C

and up to 14.5% moisture content. However, in pure nitrogen (residual oxygen less than 0.01%) the germination energy and capacity were maintained for a lesser period than in technical nitrogen but significantly longer than in air. He also noted that nitrogen gas effectively killed insects at all stages of their development within 15-20 days. He further stated that the initial quality and the history of the seeds to be preserved under nitrogen are of cardinal importance for successful maintenance of quality in general, and viability in particular, as seeds in which deterioration has already started cannot be safely stored in anoxia. Another beneficial effect of nitrogen storage is that fungi are completely inhibited in pure nitrogen and in technical nitrogen fungal proliferation is significantly retarded (De Maggio 1980). Busse (1935) stored *Populus* seeds in air and in a partial vacuum and found the life span was increased by reducing the air pressure. Barton (1961) referring to her earlier works noted that seeds of *Ulmus* species and *Pinus* species when stored under partial vacuum had extended lives. Gupta and Sood (1979), however, reported that seeds of *Dendrocalamus strictus* stored in vacuum at room temperature lost their viability faster than those stored open at room temperature. Bass (1979) reported that sorghum seeds with 4% moisture sealed in air and nitrogen germinated significantly higher than those sealed in air, but not higher than those in other atmosphere, such as argon and in vacuum. However, he noted that for both short and intermediate term storage periods, sealing in an atmosphere other than air has no advantage but there may be a long term advantage for some kind of seeds under certain storage conditions.

2.4 CONCLUSION

Tree seed can be expected to show similar behaviour to crop seed when stored and, therefore, inferences from crop seeds are most likely to be applicable to tree seeds. The review suggests that providing seeds have not been damaged in handling prior to storage, the environmental conditions during storage will have a major influence on the viability and longevity of the seed. There is abundant evidence to show that for orthodox seeds low moisture content combined with low temperature will increase seed longevity significantly. It would seem that for most practical purposes orthodox seeds are best stored at about 5-10% moisture content. This level of moisture content can be achieved and maintained by storing seeds in dehumidified areas, a method which needs elaborate equipment and is costly. Therefore, a simple method which would maintain the moisture content at constant level would be a sealed storage, such as in laminated plastic bags after conditioning the seed to low moisture content by a simple method, such as drying in a forced-draught oven. The effect of storage atmosphere is less well documented with relatively little information on tree seeds. Lowering the oxygen pressure appears to be beneficial and storage in nitrogen improves storage life and also prevents deterioration due to storage fungi and insects. The extent of differences between storage in nitrogen, carbon dioxide or in a vacuum has been investigated for mature seed of a few orthodox species but there is insufficient evidence from which to formulate a general rule applicable to a wide range of species, including tree species. This information is desirable if controlled-atmosphere storage of tree seeds is to be applied in the same way as it is currently to grain crops.

CHAPTER 3

GERMINATION EXPERIMENTS

3.1 INTRODUCTION

Maintenance of viability of a seedlot is very dependent on storage conditions. For orthodox seeds such as *P. radiata* a low temperature and low moisture content are considered beneficial for maintaining viability during storage. Variable results have emerged from studies undertaken to explore the effect of different storage atmospheres, such as carbon dioxide and nitrogen, on seed viability, especially of agricultural crops. However, no such studies have been made on *P. radiata* seeds.

This study set out to investigate the effects of different storage atmospheres on the longevity of *P. radiata* seeds. The germination capacity and the rate of germination were used as measures of the value of particular storage conditions in maintaining seed viability.

This Chapter presents and discusses the results obtained from 160 replicated germination tests. The tests were carried out on *P. radiata* seed samples stored in air, vacuum, carbon dioxide and nitrogen for a period of up to 50 weeks at a range of temperatures selected to provide both slow and rapid deterioration conditions.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Seed: *Pinus radiata* seeds were supplied by the Woods and Forests Department of South Australia. The seed was collected on Mount Crawford Forest Reserve during 1980/81 from a mature plantation. The seed had been stored in plastic bins at the seed store before being made available. Mount Crawford plantation lies at an altitude of about 430 m (1400 ft).

The seed was cleaned meticulously by hand to remove impurities. Empty seeds were eliminated from the pure seedlot by blowing air across the seed, the empty seeds being lighter separated from the full seeds and were removed. Discoloured seeds were invariably empty so these were also removed. Doubtful seed were picked up and pressure was applied with thumb and forefinger, the empty seeds collapsed and were removed.

Storage container: For this experiment it was essential that the storage container be both moisture proof and air proof. Japanese research has developed a flexible bag made from plastic laminated films with a biaxially-orientated polyamide film (nylon type), a polyvinylidene chloride film and a polyethylene film which meets these requirements (Mitsuda and Yamamoto 1980). Large laminated plastic bags, 50 x 80 cm of this type were made available by the Division of Forest Research, CSIRO. These were cut into small sheets of 11 x 18 cm and 13 x 18 cm in size. Bags were prepared by

sealing together 2 sheets of the same size with a plastic heat sealer. The seal width was 1 cm to prevent rupture and leakage when the bag was filled with gas. All bags were tested for possible leaks.

3.2.2 Method

Preparation of samples: The bulk seedlot was thoroughly mixed and passed through a Gamet seed divider to divide the seedlot into two equal parts according to the standard prescribed by the International Seed Testing Association (Anonymous 1966). This process of successive halving was repeated until a sample of 400 seeds was obtained. Two hundred samples were thus prepared.

Determination of moisture content: The moisture content of the seed lot was determined using a standard high constant temperature method (Anonymous 1976, 1980). Two samples of about 5 g each were placed in covered metal dishes and weighed. The seeds were dried in the dishes with the cover removed for one hour at 130°C and allowed to cool for 30 minutes in a desiccator when the dishes with seeds covered were weighed again. Moisture content was calculated as a percentage of the weight of the original sample by the following formula:

$$\text{Percent moisture content} = (k_2 - K_3) \times \frac{100}{(K_2 - K_1)}$$

where, K_1 is the weight in grams of the dish and its cover,

K_2 is the weight in grams of the dish, its cover and content,

K_3 is the weight in grams of the dish, cover and content after drying.

The moisture content of the seed lots was 7.54% and 8.27%, an average of 8.05%. This is well within the limits considered desirable for long-term storage of *P. radiata* seed (Holmes & Buszewicz 1958).

Storage treatment: There were three elements to the storage treatment of the seed, the atmosphere surrounding the seed, the temperature of the storage facility and the time in storage.

(a) Atmosphere: Four atmospheric conditions were chosen for experimental long term storage of the seeds. These were:

(1) air

(2) vacuum

(3) carbon dioxide

(4) nitrogen.

For storage in air a sample of 400 seeds was placed in a laminated plastic bag, 11 x 18 cm in size, and heat sealed. This ensured the bag was completely air-tight. The bag was then put into a larger laminated plastic bag 13 x 18 cm in size, and this was also sealed. The outer bag was provided as a protective cover to reduce the risk of accidental puncture of the inner bag.

For storage in a vacuum a seed sample was placed in a laminated plastic bag, 11 x 18 cm in size. Leaving 2 cm wide opening, the rest of the bag was sealed. Air was removed through the opening by a vacuum pump and the bag was completely sealed. A protective cover of an outer bag was provided and this was also sealed.

For storage in carbon dioxide the procedure was the same as for vacuum sealing except that the air in the bag was displaced by carbon dioxide gas from a cylinder before sealing.

For storage in nitrogen gas the same procedure was followed so that the air in the bag was replaced by nitrogen before sealing. Fifty samples of each of the four storage atmospheres were prepared and labelled (Plate 3.1).

Tests to check the composition of the atmosphere within the air-sealed bags after a period of storage were made after the main germination tests were completed. Bags of seeds were stored at each of four temperatures: 5, 15, 25 and 35°C - for 10 weeks with air as the storage medium. Three bags were available for sampling at each of the four temperatures at 5 weeks and at 10 weeks from the commencement of the storage period. Seed samples of 400 seeds were placed in identical laminated plastic bags to those used in the main experiments and air sealed. At 5 and 10 weeks subsequent to storage treatment the atmosphere within each bag was sampled and its composition analysed by gas chromatography technique to determine the

Plate 3.1: A sample of seed in an atmosphere of nitrogen sealed in laminated plastic bag.

change in carbon dioxide and oxygen levels. Two hundred microlitre samples were taken from each bag by means of a syringe through a self-sealing rubber valve. The sample was injected into a gas chromatograph apparatus, Aerograph 200, with a thermal conductivity detector. The amount of oxygen present in the sample was calculated from the gas chromatogram. A determination was made for each of 3 replicates for each storage treatment. The same procedure was followed to measure the amount of carbon dioxide gas in the sample except that a gas chromatograph with a flamisation detector was used.

(b) Temperature: Five lots of sealed samples, each lot consisting of ten samples of each atmosphere were stored, one lot at each of 5, 15, 25, 35 and 45°C temperatures. Electric ovens were employed to store seed at 35 and 45°C and constant temperature rooms used for storage at 5°, 15 and 25°C. Temperature fluctuation of the storage was ± 1°C. An extra sample in each treatment was stored to make allowance for damage or loss.

(c) Storage time: A set of samples, each of air, vacuum, carbon dioxide and nitrogen was removed from storage every 5 weeks for 50 weeks.

In summary, the experiment involved three elements:

Storage atmosphere : 4

Storage temperature: 5

Storage time: 11, including time 0.

After only 10 weeks it was evident that seed stored at 45°C had lost germination capacity very rapidly irrespective of storage atmosphere and this treatment was subsequently abandoned and does not appear as part of the final analysis. The final analysis is therefore concerned with 4 x 4 x 11 experimental storage combinations.

Germination testing: Seed was tested routinely for germination as it was removed progressively from storage. Each sample of 400 seeds stored in the sealed bag was divided at random into 10 lots of 40 seeds to give 10 replications for each germination test.

Germination dish: Approximately 5 g of vermiculite was spread over a 9 cm diameter petri dish and a filter paper, Whatman No. 50, was placed on top of it. Petri dishes prepared in this way were sterilised in an autoclave at 121°C under a pressure of 150 kilopascals for 30 minutes. Sterilised water (15 ml) was added in each dish. The sample of 400 seeds taken out of storage was surface sterilised in a vacuum for 5 minutes with 1% sodium hypochlorite solution, then washed with sterile water. A replicate of 40 seeds was counted out and placed uniformly spaced in a prepared petri dish. Petri dishes were covered, labelled, then placed in a germination cabinet. All preparation of the germination dishes was carried out in a sterile room.

Germination condition: An enclosed germination cabinet was used for the seed germination tests. Seeds were germinated at a constant temperature of 20°C with 8 hours of light daily as recommended by the International Seed Testing Association (Anonymous 1976). Light in the germination cabinet was provided by eight 40 watts white fluorescent tubes. The light intensity was measured as 10 microeinstein in the centre of the cabinet and this level of illumination was maintained throughout the experimental period.

The germination test period was initially set at 28 days following the International Seed Testing Association's prescription but as a significant number of seeds was still germinating the test period was extended to 35 days.

Germination counts were made daily for 28 days and thereafter every alternate day for 7 days. A seed was considered to have germinated when the root was more than 3 cm in length. Seeds germinating normally were counted and removed at this point. The germination medium was kept moist by adding sterilised water whenever necessary to maintain the humidity inside the petri dish. At the termination of the test period, the remaining seeds in the petri dish were squashed to determine if they were viable or dead.

In summary, the germination test involved the following elements:

Germination dish:	9 cm covered petri dish.
Number of seed per dish:	40.
Number of replications:	10.
Sterilisation:	1% sodium hypochlorite in vacuum for 5 minutes.
Germination medium:	Whatman No. 50, 9 cm paper on top of vermiculite.
Moisture:	15 ml of sterilised water initially, added when necessary.
Temperature:	20°C constant.
Light:	White fluorescent tubes, with 10 microeinstein intensity 8 hours daily.
Period of test:	35 days.
Germination count:	Daily for 28 days, thereafter alternate days.
Definition of germination:	Minimum radicle protrusion 3 cm.

3.3 DATA EXPRESSION AND ANALYSIS

The total number of seeds germinating and the rate of germination are generally taken as the parameters for comparative germination studies (Goodchild and Walker 1971). The total number of seeds germinating under a set of conditions is generally expressed as a percentage of the total number of viable seeds in a sample i.e. germination percent. Cumulative germination percentage may be defined as the cumulative number of germinated seeds expressed as a percentage of the total viable seeds. Germintion rate, or the time taken by seeds to germinate, can be expressed in different ways e.g. time taken to reach a nominated percentage of the germination capacity of the seedlot, or percentage of seeds germinated after a certain time period, or by computing the germination times of all individual seed, the coefficient of velocity as developed by Kotowski (1926).

The mean of the percentage germination of each of 10 replications was taken as the percentage germination for each treatment. The cumulative germination percentages were plotted against the corresponding number of days since sowing to show the course of germination for some storage treatments. Mean germination percentages were plotted against storage time periods under slow and rapid deterioration conditions for each storage atmosphere to show the course of deterioration over 50 weeks. For the purpose of this study, the following formula proposed by Kotowski (1926) for measuring the rate of germination was used:

$$\text{Coefficient of velocity} = \frac{A_1 + A_2 \ldots\ldots + A_x}{A_1T_1 + A_2T_2 \ldots + A_xT_x} \times 100$$

where, A = number of seeds germinated

T = the number of days after sowing corresponding to A.

In other words, the numerator is the sum of daily counts and represents the ultimate germination, and the denominator is the summation of germination counts, each weighted by number of days after commencement of the test. The resulting quotient is multiplied by 100. This method approximates the integral of germination periods of individual seeds. This formula was chosen because it has considerable merit for comparing different lots of seeds, or different treatments, providing a standard pattern of counting frequency is always used (Czabator 1962). Coefficient of velocity of germination was calculated for the time periods of 10, 20, 30, 40 and 50 weeks subsequent to storage treatment and a control. A high value for coefficient of velocity of germination denotes;

(a) increased number of seeds germinating, and

(b) decrease in time of germination.

The graphical method of presenting germination results is very informative although not statistically tractable (Heydecker 1966). Conventionally therefore the results of germination tests are interpreted by statistical analysis (Czabator 1962). Statistical analysis of germination data was carried out. For

analysis of variance, the germination percentage of each of the 10 replications for each treatment was used to calculate the means for each of the following factors and their interaction:

Factor 1: Atmosphere at 4 levels - air, vacuum, carbon dioxide and nitrogen

Factor 2: Temperature at 4 levels - 5, 15, 25 and 35°C.

Factor 3: Time at 11 levels - 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 weeks and a control (0 weeks)

Before analysis, all the percentage data were transformed into arcsin because of the binomial nature of data. Data were analysed by using a computer programme, GENSTAT, (Alvey *et al.* 1977). The general treatment means were also compared by using least significant difference (LSD) test or 't' test (Steel and Torrie 1960). The 'F' and 't' distributions were taken from the tables of Fisher and Yates (1949). For the difference between two means to be significant at any level of significance, the observed differences must exceed the LSD value.

The values of coefficient of velocity of germination were also statistically analysed. For analysis of variance, the value of coefficient of velocity of each of the 10 replications for each treatment was used to calculate the means for each of the following factors and their interaction:

Factor 1:Atmosphere at 4 levels - air, vacuum, carbon dioxide and nitrogen

Factor 2: Temperature at 4 levels - 5, 15, 25 and 35°C

Factor 3: Time at 6 levels - 2,4,6,8,10 weeks and a control (0 weeks)

GENSTAT was used for analysing the data. Differences between general treatment means were compared by the method of LSD test.

3.4 RESULTS

Periodic checking of the samples placed in storage revealed that the carbon dioxide had been completely adsorbed by the seeds, creating much the same effect in the bag as in the vacuum samples, a phenomenon previously described by Mitsuda *et al.* (1972). The adsorption of the carbon dioxide took place much faster at the higher temperatures.

Two criteria were used to compare the germination response of seeds stored under the four different conditions. They were:

(a) germination percentage after 35 days, and

(b) germination rate.

3.4.1 Germination percentage after 35 days

The results of the germination tests (Table 3.1) were analysed using analysis of variance technique. The variance ratio (F value) for all the main factors - storage atmosphere, temperature and time - were significant at the .01 probability level (Table 3.2). The variance ratio contributed by atmosphere (150.48) was much higher than by the other two factors - temperature (41.04) and time (46.29) indicating that the influence of storage atmosphere was of more significance in the maintenance of viability than temperature and time period. The first order interactions were also found to be significant at the .01 probability level. The second order interaction (Atm. x Temp. x Time) was not, however, significant at the .05 probability level. From Table 3.1 it can be observed that nitrogen storage was the best treatment for maintaining the viability of the seeds followed by carbon dioxide, a vacuum, and air, in that order. The arcsin transformed means of germination percentage for nitrogen (74.18) and carbon dioxide (71.88) were significantly different from each other and from the other two but vacuum (68.50) and air (68.20) were not different from each other (Table 3.3).

There were some variations or fluctuations in the germination results at earlier test periods (Table 3.1). This may have been due to the effect of the fungicides which were used to tackle the problem of infection in the germination dish. The application of fungicide was later abandoned and more stringent

Table 3.1 Mean percentage germination pertaining to each germination test for all storage treatments

Storage condition		Storage time in weeks										
Atmosphere	Temperature (°C)	0	5	10	15	20	25	30	35	40	45	50
Air	5	94.60	92.60	90.10	85.67	83.00	91.00	88.90	85.29	86.78	85.00	84.20
	15	94.60	90.30	87.70	89.30	83.10	88.30	86.75	86.43	85.78	82.00	82.70
	25	94.60	91.50	86.60	82.22	84.30	86.80	87.70	84.00	79.13	77.89	76.11
	35	94.60	89.70	82.20	78.89	87.40	84.40	84.78	81.60	72.40	71.11	67.13
Vacuum	5	94.60	93.30	86.30	83.00	85.60	86.00	90.12	83.30	88.11	86.56	86.43
	15	94.60	88.70	88.30	88.28	90.40	86.30	86.01	84.20	85.50	84.22	85.60
	25	94.60	92.10	78.70	84.90	87.60	89.50	86.20	84.10	82.60	83.50	83.30
	35	94.60	92.20	80.50	79.44	80.40	85.70	78.44	80.38	72.80	75.56	73.70
Carbon dioxide	5	94.60	87.44	84.67	89.60	92.37	92.27	90.85	89.25	89.87	90.14	90.30
	15	94.60	86.50	87.50	91.00	91.06	91.28	88.33	91.11	89.78	91.62	90.00
	25	94.60	87.89	84.40	90.70	91.75	93.75	91.11	89.89	88.50	88.62	88.44
	35	94.60	85.00	82.60	91.20	92.00	89.87	89.40	89.10	88.50	87.70	81.80
Nitrogen	5	94.60	94.44	92.90	92.70	92.00	92.27	95.62	91.75	91.84	91.42	89.53
	15	94.60	92.22	92.00	91.89	91.80	94.80	92.70	92.22	92.78	90.63	89.75
	25	94.60	91.62	93.33	92.80	89.33	93.11	91.22	90.75	90.87	90.79	89.12
	35	94.60	89.33	92.90	92.33	92.56	92.00	90.22	89.67	89.75	91.12	86.78

Table 3.2 Summarised analysis of variance of germination percentage data presented in Table 3.1

Source of Variation	Degrees of freedom	Mean squares+	Variance ratio (F)
Atmosphere	3	3609.36	150.48**
Temperature	3	984.42	41.04**
Time	10	1110.30	46.29**
Atmosphere x Temperature	9	86.51	3.60**
Atmosphere x Time	30	188.54	7.60**
Temperature x Time	30	67.37	2.80**
Atmosphere x Temperature x Time	90	30.13	1.25NS
Residual	1428	23.99	

N.B. + = Arcsin transformed value

NS = Not significant at P = .05

** = Significant at P = .01

Table 3.3 General germination mean[+] values for different storage atmosphere and temperatures.

Mean values which do not have the same affix are significantly different within the column at .05 level according to LSD test.

Storage atmosphere				Storage temperature °C			
Air	Vacuum	Carbon dioxide	Nitrogen	5°	15°	25°	35°
68.20[a]	68.50[a]	71.88[b]	74.18[c]	71.89[a]	71.61[a]	70.68[b]	68.58[c]

[+] Arcsin transformed value

Standard error of difference between means = .330

Least significant difference value = .65

precautions were taken, including counting the germinants in a sterile box fitted with ultra violet light and periodic fumigation of the germination cabinet, to minimise the problem of fungal infections.

At 5°C the percentage germination for seed stored in nitrogen was 89.53% at the end of the 50 week storage period which was little different from that for seed stored in carbon dioxide (90.30%). Although both of these treatments were better than vacuum (86.43%) and air (84.20%), all four treatments demonstrate the value of low temperature storage of seed. Loss of viability of the seeds stored at 5°C was very gradual over the period of 50 weeks and the original high level of germination was maintained. However, even as early as 25 weeks some different trends were apparent in the treatments. Air storage began to reveal a slightly poorer germination at this stage. Seed stored in a vacuum began to show a slight decline in germination percentage at 30 weeks. However all of these trends can be viewed as minor when seen in the context of storage at higher temperatures when physiological deterioration of the seed could be expected.

Higher storage temperatures of 15°C and 25°C had no marked effect on germinability of seeds when stored in an atmosphere of nitrogen and carbon dioxide. When stored in a vacuum, the germination percentage decreased to 86.01% and 86.20% at 15°C and 25°C respectively at 30 weeks and at 50 weeks there was further decline in the 25°C treatment. In air storage an even more

pronounced effect can be seen, especially at 25°C at which temperature germination percent declined rapidly after 35 weeks in storage.

Under conditions of accelerated aging at 35°C there were marked differences in deterioration of seeds among different storage atmospheres, especially at the 50 week period. From Table 3.1 the beneficial effect of nitrogen storage compared to other storage atmospheres was very clear. For example, nitrogen storage retained 86.78% germination at the end of the 50 week period while carbon dioxide storage gave 81.80% germination. The extent of seed deterioration was evident in the case of vacuum storage which gave 73.70% germination while in air storage germination had decreased to 67.13%. In nitrogen storage, the germination of over 90% was maintained up to 30 weeks whereas in carbon dioxide that level of germination was not maintained beyond 20 weeks. In vacuum, the percentage germination is over 90% was maintained only up to 5 weeks and thereafter the decrease was rapid, and more so at the later periods. In air storage, even after 5 weeks the germination fell below 90% and in the later period of storage, the drop in germination percent was quite marked. Figure 3.1 shows the loss in germination percent as compared to time 0 week for all treatments for 50 weeks period. It can be seen that loss in germination percent under all storage temperatures was highest in an atmosphere of air.

Statistical analysis of the means of storage temperature show that there was no significant difference between 5°C and 15°C at

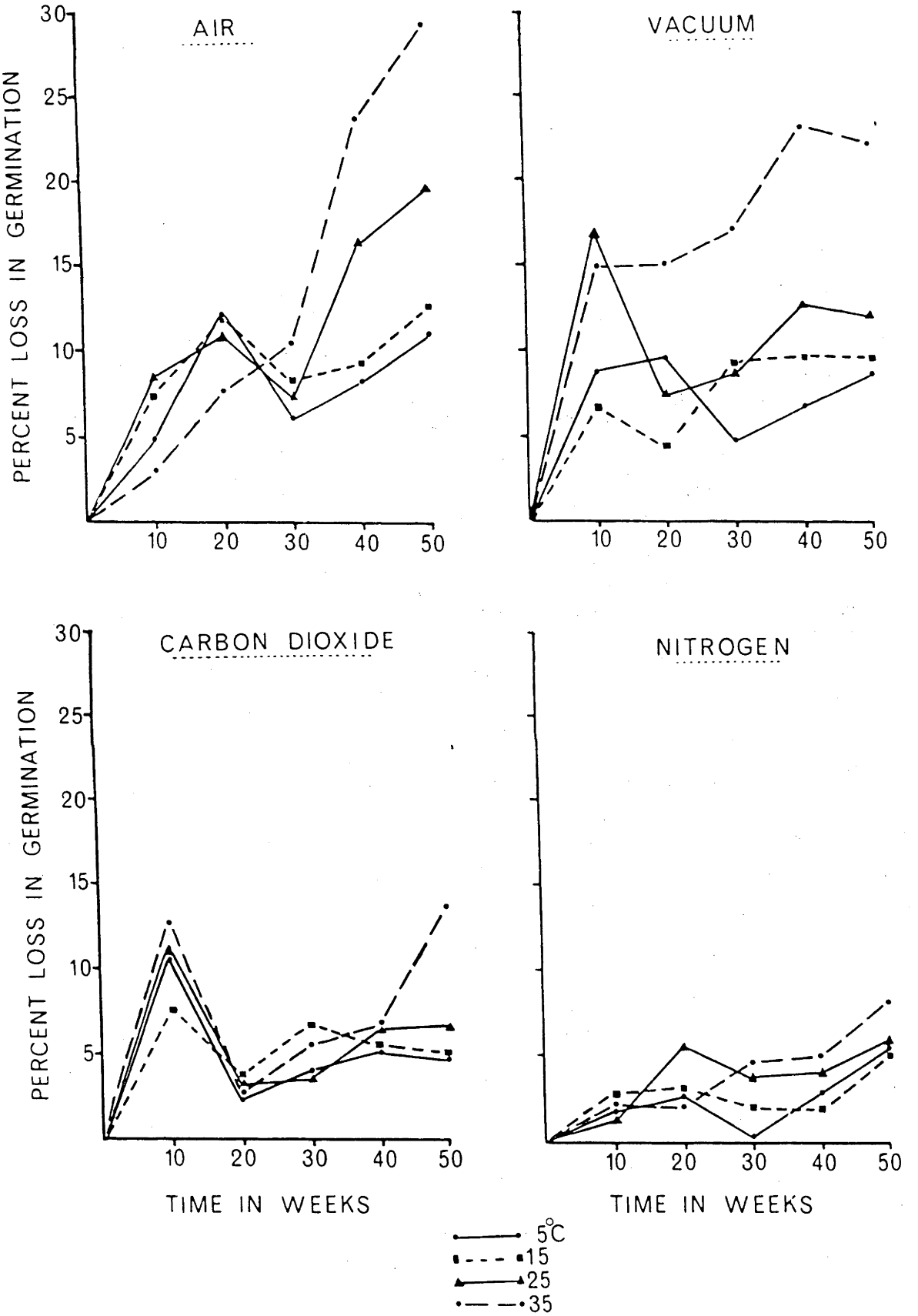

Figure 3.1 Comparative loss in germination percent as compared to time 0 (control) for each of the four storage temperatures for each storage atmosphere for 50 weeks.

the .05 probability level (Table 3.3). However, means for other temperatures show significant differences among themselves at the .05 probability level.

Percentage germination in all treatments decreased during the 50 week storage period as compared to the initial germination percent (control) of 94.64%. Table 3.4 gives statistical comparisons of the germination mean value for each storage period. The mean values at all storage periods were significantly different from the control at the .05 probability level. The mean values for the 5 and 25 weeks periods were not significantly different (P = .05) from each other whereas all other mean values following 25 weeks period were significantly different compared to the mean value for the 25 weeks period.

Figure 3.2 shows the comparative course of final germination percent for each storage atmosphere under the conditions of storage over a period of 50 weeks. It can be seen that under both low and high storage temperatures the seeds in nitrogen deteriorated the least of all the storage atmospheres.

3.4.2 Germination rate

Table 3.5 shows the germination rate in terms of coefficient of velocity of germination calculated for all storage conditions for time periods of 10, 20, 30, 40, 50 weeks and a control (week 0). Statistical analysis of the data shows that the variance ratio for all main factors - atmosphere, temperature and time were highly

Table 3.4 General germination mean values for different storage time periods.

Mean values which do not have the same affix are significantly different at .05 level according to LSD test.

Storage time in weeks	Germination mean +
0	76.77^{a}
5	72.90^{be}
10	69.57^{cgh}
15	70.35^{cdfg}
20	70.79^{df}
25	72.35^{e}
30	71.10^{f}
35	69.45^{gh}
40	68.93^{hi}
45	68.29^{i}
50	67.10^{j}

+ Arcsin transformed value

Standard error of difference between means = .548

Least significant difference value = 1.07

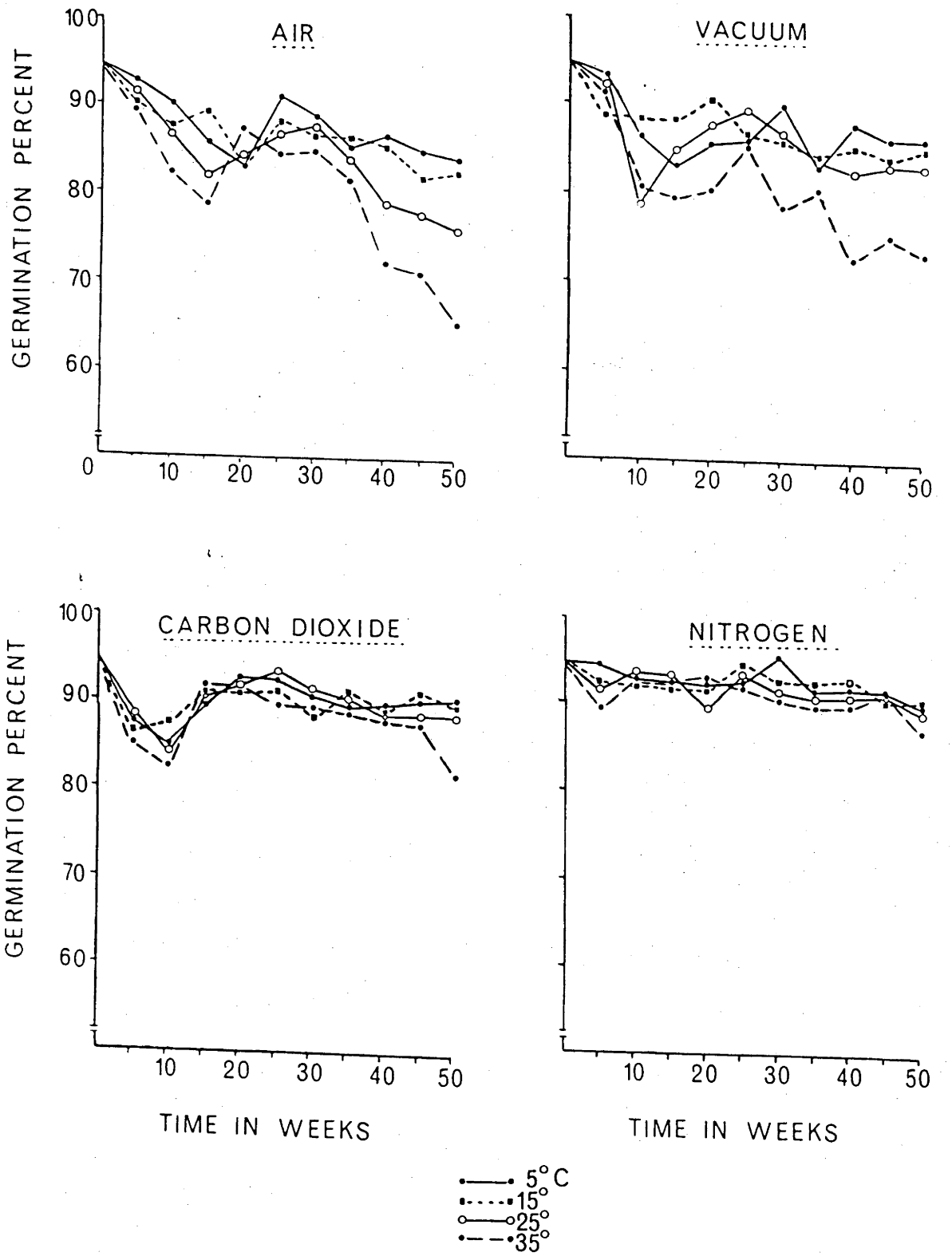

Figure 3.2 Comparative course of final germination percent for each of the four storage temperatures for each storage atmosphere for 50 weeks.

Table 3.5 Mean coefficient of velocity of germination pertaining to germination tests at different time period for all storage conditions

Storage Condition		Storage time in weeks					
Atmos-phere	Tempera-ture (°C)	0	10	20	30	40	50
Air	5	6.90	6.67	5.35	5.77	5.80	5.34
	15	6.90	6.48	5.49	6.16	5.95	5.49
	25	6.90	5.71	5.37	5.82	5.42	5.15
	35	6.90	6.11	5.15	4.98	4.67	4.04
Vacuum	5	6.90	6.28	6.04	5.92	5.71	5.46
	15	6.90	6.42	6.12	6.04	5.93	5.53
	25	6.90	5.68	6.15	5.98	5.80	5.29
	35	6.90	5.54	5.26	5.09	4.67	4.34
Carbon dioxide	5	6.90	5.78	6.28	6.07	6.04	5.84
	15	6.90	5.74	6.33	6.09	6.10	6.02
	25	6.90	5.49	6.15	5.87	5.75	5.84
	35	6.90	5.67	5.52	5.00	4.92	4.88
Nitrogen	5	6.90	6.20	6.42	6.37	6.31	6.29
	15	6.90	6.33	6.44	6.34	6.32	6.23
	25	6.90	6.26	6.45	6.30	6.19	6.10
	35	6.90	6.45	6.13	5.80	5.48	5.17

significant at a probability level of .01 (Table 3.6). The time factor contributed a much higher variance ratio (481.19) than the two other factors - temperature (283.70) and atmosphere (151.25) indicating the significance of time in the maintenance of rate of germination. The first order interactions of all combinations and the second order interactions were also significant at the .01 probability level although compared to the main factors these variance ratios were modest.

From Table 3.5 it can be observed that seed stored in nitrogen not only maintained a higher percentage germination but also a high rate of germination. Air storage was the least effective in reducing the deterioration of the germination rate. Statistical comparison of the means of storage atmospheres show that they were significantly different from one another at the .05 probability level (Table 3.7). Nitrogen storage provided a highest mean value (6.30) as against a lowest value (5.77) for air storage, with carbon dioxide (5.96) and vacuum (5.87) intermediate.

The rate of germination was highest for seeds stored in nitrogen, progressively less for seed stored in carbon dioxide, vacuum and air, in that order. The rate of germination in each of the storage atmospheres decreased also as storage temperature was increased from 5°C through 15°C, 25°C to 35°C.

Statistical analysis of means of germination rate at the four storage temperatures shows that the means were significantly

Table 3.6 Summarised analysis of variance of coefficient of velocity of germination presented in Table 3.5

Source of variation	Degree of freedom	Mean squares +	Variance ratio (F)
Atmosphere	3	12.64	151.25**
Temperature	3	23.72	283.70**
Time	5	40.24	481.19**
Atmosphere x Temperature	9	0.46	5.50**
Atmosphere x Time	15	2.19	26.27**
Temperature x Time	15	2.37	28.42**
Atmosphere x Temperature x Time	45	0.17	2.04**
Residual	786	0.08	

N.B. + = Arcsin transformed value

** = Significant at P = .01

Table 3.7 General means of coefficient of velocity of germination of different storage conditions and time periods.
Mean values which do not have the same affix are significantly different within the block at .05 level according to LSD test.

Storage atmosphere			
Air	Vacuum	Carbon dioxide	Nitrogen
5.77^{a}	5.87^{b}	5.96^{c}	6.30^{d}

Storage temperature °C			
5	15	25	35
6.15^{a}	6.22^{b}	6.02^{c}	5.52^{d}

Storage time in weeks					
0	10	20	30	40	50
6.90^{a}	6.05^{b}	5.92^{c}	5.85^{d}	5.69^{e}	5.44^{f}

Standard error of difference between means for
(a) atmosphere and temperature = .026
(b) time = .032
Least significant difference value for
(a) atmosphere and temperature = .051
(b) time = .063

different from one another at the .05 probability level (Table 3.7). Table 3.7 provides statistical comparison of the mean values for germination rate of each storage period including time zero week (control). It can be seen that the mean values of all storage time periods were significantly different from time zero (control) and also from each other at the .05 probability level.

Figure 3.3 shows the comparative course of germination rate over a period of 50 weeks of different storage atmospheres under slow (low temperature) and rapid (high temperature) deterioration conditions, illustrating how low temperature nitrogen storage retained a better germination rate than any of the other storage atmospheres.

The time taken for germination to begin for the different treatments is shown in Table 3.8. At 5°C, the time taken for germination to begin was 12 days for both nitrogen and carbon dioxide storage while it was 13 days for both vacuum and air storage after 50 weeks in storage. The time taken for the first germinants to appear at higher temperatures of 15°C and 25°C was similar to that at low temperature.

Figure 3.4 shows the comparative course of germination at four storage temperatures for each of the storage atmospheres at 50 weeks. It can be seen that the seeds stored in nitrogen germinated earlier and faster than those stored in air at all storage temperatures.

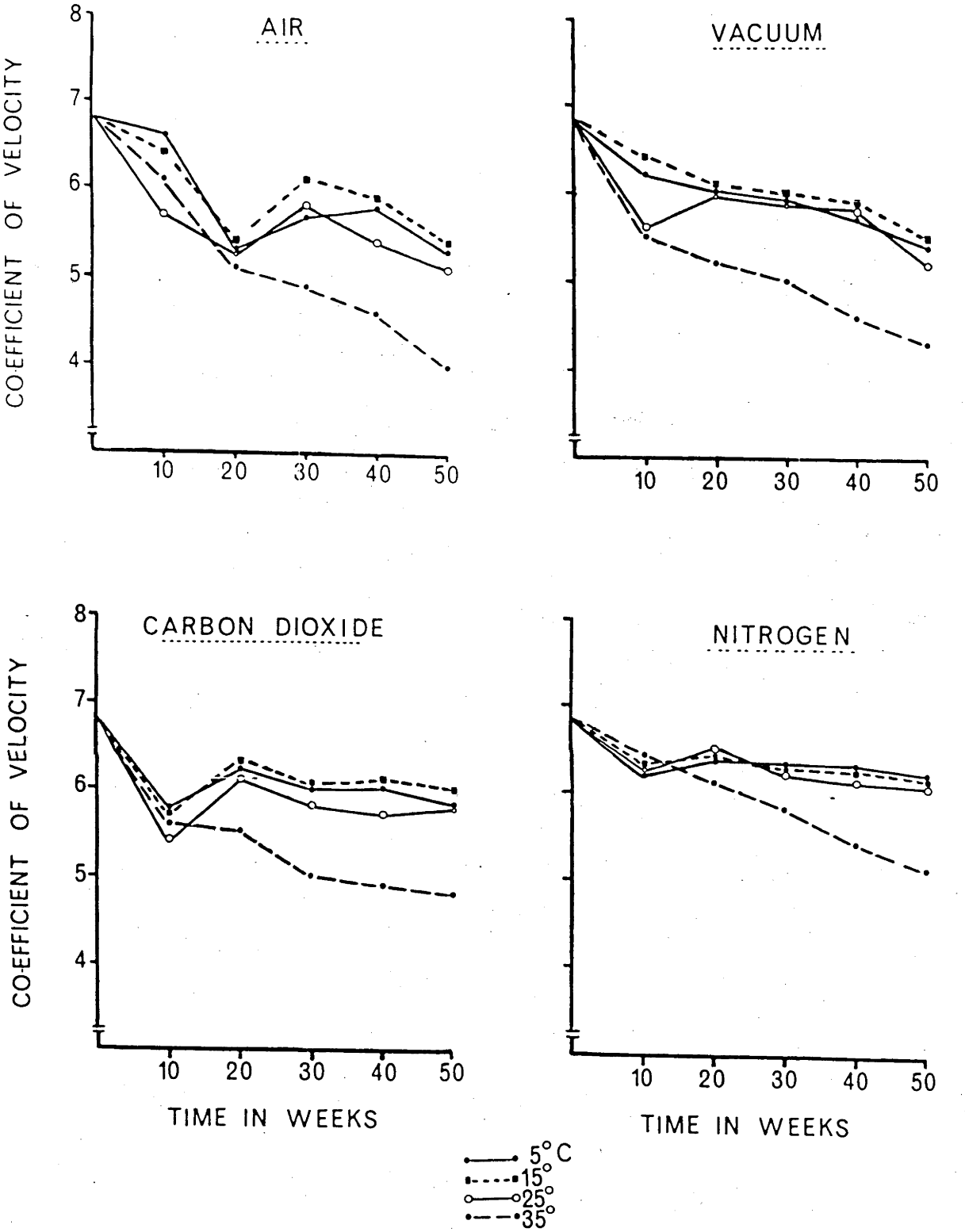

Figure 3.3 Comparative course of germination rate for each of the four storage temperatures for each storage atmosphere for 50 weeks.

Table 3.8 Time (days) taken for germination + to begin at different temperatures for seed stored under different conditions for varying time periods.

Storage conditions		Storage time period in weeks					
Atmosphere	Temperature (°C)	0	10	20	30	40	50
Air	5	11	11	11	12	12	13
	15	11	11	11	12	12	13
	25	11	11	12	12	13	13
	35	11	11	13	14	15	16
Vacuum	5	11	11	12	12	12	13
	15	11	11	12	12	12	13
	25	11	11	12	12	12	13
	35	11	11	13	13	14	15
Carbon dioxide	5	11	11	12	12	12	12
	15	11	11	12	12	12	12
	25	11	12	12	12	13	12
	35	11	12	13	14	13	15
Nitrogen	5	11	12	12	12	12	12
	15	11	12	11	12	12	12
	25	11	12	12	12	12	12
	35	11	12	12	13	13	13

+ germination = when the radicle exceeds 3 cm in length.

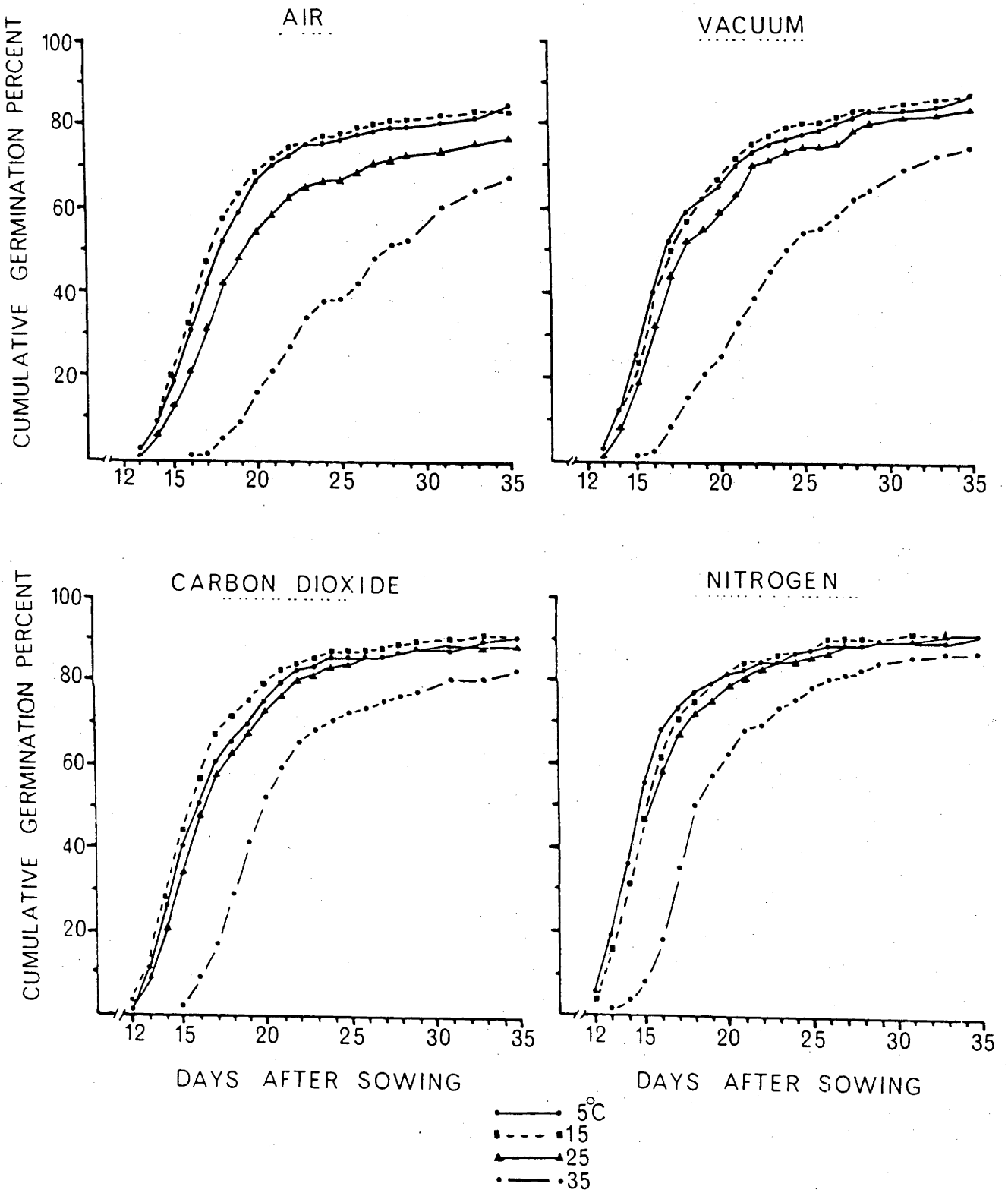

Figure 3.4 Comparative course of germination at four storage temperatures for each of the storage atmospheres at 50 weeks.

3.4.3 Composition of air in the storage bags

The results of gas analysis are presented in Table 3.9. They show that after 5 weeks storage the oxygen content in the bag decreased as the storage temperature increased. At 5°C the oxygen content in the bag was slightly decreased to 20.40% while at 35°C it was decreased to 18.04%. Though the decrease in oxygen content was varying from 18.04 to 20.04% at different temperatures, increase in carbon dioxide concentration was almost to the same level of 0.18% at all temperatures. At 10 weeks storage period there was further decrease in oxygen content especially at higher temperatures. It was decreased to 16.02% at 35°C while at 5°C the decrease was minimal. It can also be seen that there was no further increase in carbon dioxide content at 10 weeks period. Instead it decreased to 0.13% and 0.15% at 5°C and 35°C respectively. In a closed system the effect of respiration of the stored seeds would be to decrease the proportion of oxygen in the atmosphere and increase the amount of carbon dioxide. The oxygen level fell as expected but contrary to expectations the carbon dioxide level remained static. To explain this it is suggested the excess carbon dioxide may have been adsorbed by the seed in the same way as in the seed sealed in carbon dioxide.

3.5 DISCUSSION

Carbon dioxide was rapidly and completely adsorbed by the seeds during storage. This phenomenon of adsorption has also been reported in grain seeds such as rice, wheat, corn by Mitsuda *et al.* (1973) who suggested four possible reasons for it:

Table 3.9 Changes in gaseous composition with time in air sealed storage at different temperatures

Time in weeks	Storage temperature (°C)	Oxygen percent	Carbon dioxide percent
0	5	21.0	0.03
	15	21.0	0.03
	25	21.0	0.03
	35	21.0	0.03
5	5	20.40	0.18
	15	20.32	0.19
	25	19.59	0.15
	35	18.04	0.18
10	5	20.04	0.13
	15	19.20	0.10
	25	18.04	0.10
	35	16.02	0.15

(a) dissolution of carbon dioxide into water,

(b) dissolution of carbon dioxide into fats and oils,

(c) diffusion of carbon dioxide into porous tissues of the seeds, and

(d) biological fixation of carbon dioxide by tissues of the seeds.

However, it was concluded that the most important factor is the diffusion of carbon dioxide into the porous parts of the seeds by sorption, a phenomenon similar to sorption of gases by charcoal and silica gel. The carbon dioxide sorbed by porous tissues of seed is considered to remain in solid solution and may be completely reversed (desorption) when the seed is allowed to stand in air. It is doubtful whether this phenomenon as such has any direct effect on viability and vigour of seeds but has practical application in the development of a new technique for skin-packaging, the Carbon dioxide Exchange Method (CEM) (Mitsuda *et al.* 1973). This method is useful in that it makes the packed substance compact, ensures safe transport and eliminates oxygen from the package substance. Much the same advantages would be obtained by packing seed in this way. Storage space would be saved, and packaged seed could be transported over long distances safely.

The result revealed that seeds sealed in air gave lowest viability as compared to those in vacuum, carbon dioxide and nitrogen which gave the highest viability during 50 weeks storage period. This agrees with Harrison's (1966) result on lettuce seeds. He found that lettuce seeds, 6% moisture content stored at 18°C for 3

years gave mean germination of 78% in nitrogen and carbon dioxide, 77% in vacuum and 57% in sealed air as compared to 48% in open air and 8% in pure oxygen. The lower viability of dry seeds sealed in air is no doubt due to the deleterious effect of oxygen present in the air. Oxygen not only affects viability by its presence or absence but also by its increased level as Roberts and Abdalla (1968) found that more deleterious effects of oxygen were obtained on increasing the oxygen level from nominal 0% to 21%. They also found that a partial pressure of 21% was almost as deleterious as 100% oxygen at atmospheric pressure, irrespective of other conditions of storage. This observation was also reported earlier by Peterson *et al.* (1956) for wheat grains in which by successive increasing partial pressure of oxygen from 0.2% to 21%, the viability was reduced from 86% to 7%.

In the present study though the mean percent germination values for nitrogen (74.18%) and sealed air (68.20) were distinct and statistically significant, but the difference was not as large as one would expect considering the deleterious effect of oxygen. This may be explained by the fact that there is a change in composition of gaseous environment in hermetically sealed air with time. As time passes, the partial pressure of oxygen decreases and that of the carbon dioxide increases due to the respiration of the seeds and associated microflora. This was shown in the present study, by the results of gas analysis. Roberts and Abdalla (1968) showed that pea seeds with 18.4% moisture content sealed in air and stored at 25°C caused a more or less linear decrease in oxygen (from 21% to 1.4%)

and an increase in carbon dioxide (from 0.03 to 12%) in closed atmosphere in 11.3 weeks, the time taken to reach 50% viability. Because of these gas changes, they found the values of the half viability period for sealed air was much higher (82.3 days) than that of constant air atmosphere (56 days) but much lower than constant nitrogen atmosphere (91 days). Therefore, the rate of deterioration of seeds was slower in hermetically sealed air than in replenished air. These results emphasize the benefits of sealed storage as not only is the relative humidity of the air surrounding the seed kept at a desirable level but also the partial pressure of oxygen in that air will decline with storage time.

At the low storage temperature of 5°C, seeds stored in all four storage atmospheres - nitrogen, carbon dioxide, vacuum and air maintained the original high level of viability with only a slightly lower level in vacuum and air after a period of 50 weeks. At higher storage temperatures of 15°C and 25°C there was marked effect on viability in seeds sealed in air and less so in vacuum but not in nitrogen and carbon dioxide. This suggests that at low moisture content (8%) and a low temperature (5°C), the rate of respiration in the seed is very low, and here the presence of oxygen has little effect on viability of seed. At higher temperature, especially at 25°C the rate of seed respiration will increase markedly and here the presence of oxygen has a marked effect on seed viability, even at a low moisture content.

The results of even more accelerated ageing in seed stored at 35°C revealed more marked differences in deterioration in seeds among the four storage atmospheres, especially between nitrogen and air sealed. Even at this temperature seeds in nitrogen maintained a high viability. However, at the end of 50 weeks the loss of viability in air storage (29.04%) was more than 3 times to that of nitrogen (8.27%) and about twice that of carbon dioxide (13.53%). For the same length of time at a low temperature of 5°C, the loss in percentage germination in air (10.99%) was 2 times compared with nitrogen (5.47%) and carbon dioxide (4.55%).

The results also support the theory behind the methodology of the study which assumed ageing of the seed would be accelerated at higher temperatures. Delouche and Baskin (1973) assumed also that in artificial ageing of seeds the process of deterioration is similar to those of normal conditions only the rate of deterioration is greatly increased.

This study has also further revealed that seeds sealed in nitrogen maintained not only a higher level of germination but also a higher rate of germination as compared to those in carbon dioxide, vacuum and air sealed during 50 weeks storage period at all temperatures. Some deterioration in germination energy was found to occur as the rates for all treatments were less at 50 weeks than they were at week 0. Once again nitrogen proved to be more effective in reducing the deterioration in germination rate than other storage atmospheres. The lower rate of germination in air-sealed storage

may again be attributed to the deleterious effect of the oxygen present in air. Even at low temperature it seems that the presence of oxygen is detrimental to the maintenance of a high germination rate because there was a marked difference in germination rate between air-sealed and nitrogen storage. The presence of oxygen had a less marked effect on viability of seed at low temperature. The results agree well with the report that samples of seeds having the same germination may have reached different levels of deterioration (Harrison 1966). Germination rate has been a more sensitive indicator of seed deterioration in storage.

It is generally believed that the deleterious effect of oxygen is due to a stimulation of respiration. However, other evidence does not seem to reconcile with this view. Roberts and Abdalla (1968) showed that even at higher temperature of 60°C oxygen had a markedly deleterious effect on viability. They argued that it is improbable respiration of the seeds would continue to function at such a high temperature. The deleterious effect of increased oxygen pressure on dry seed may be due to other oxidative processes (Roberts and Ellis 1982). Whatever the process by which oxygen affects the viability of seeds the cause of loss of viability is attributed to sub-cellular damage in the seed (Roberts and Ellis 1982). It has been suggested by Villiers and Edgecumbe (1975) that during storage sub-cellular damage occurs continuously in all seeds at a rate dependent on moisture content and temperature. In dry, orthodox seeds metabolic processes occur at a low rate and there is little repair and turnover of damaged components. The damage utlimately

accumulates to catastrophic levels, at which point the seed is incapable of germinating i.e. loses viability. They further envisaged that in fully hydrated orthodox seeds, the repair and turnover mechanisms are active and so damage is not allowed to accumulate. Hence, the seeds are able to maintain viability for quite a long time. However, Ibrahim and Roberts (1983) showed that in this hydrated situation oxygen is essential for respiration to be able to sustain the normal metabolism so that repair and turnover mechanisms are active.

It is clear, therefore, that there is an interaction between moisture content and partial pressure of oxygen. It would seem there is a point in the moisture content level above which the unfavourable relationship of partial pressure of oxygen would change into a favourable one in orthodox seed. Ibrahim and Roberts (1983) believe that this point may vary from species to species.

In conclusion, the viability of dry orthodox seeds is reduced in the presence of oxygen because sub-cellular deterioration occurs at a faster rate than in its absence. Therefore, storage of seeds in the absence of oxygen, such as in nitrogen and carbon dioxide could reduce the rate of sub-cellular deterioration. In contrast, fully hydrated orthodox seeds cannot be stored in atmospheres, such as nitrogen or carbon dioxide because sub-cellular

deterioration is enhanced at a rate dependent on moisture content in the absence of oxygen. In such situation, oxygen is essential for respiration to sustain normal metabolism, such as repair and turnover mechanism to prolong the viability period. Seeds of *Pinus radiata* can be stored better in oxygen free atmosphere at lower temperatures and moisture content. For long term storage, atmosphere of nitrogen proved to be better than carbon dioxide and vacuum.

3.6 SUMMARY OF RESULTS

One of the aims of this study was to compare the effects of sealed storage atmospheres of air, vacuum, carbon dioxide and nitrogen on the germinability and rate of germination of seeds stored at low moisture content. Consistent with the aim, the findings are summarised as follows:

1. Analysis of variance of the means showed the variance ratios (F value) for all main factors - storage atmosphere, temperature and time - were significant at the .01 probability level.
2. Losses in germination percent and germination rate were highest for seeds (8% moisture content) stored in an atmosphere of air under all storage temperatures.
3. Seeds stored in an atmosphere of nitrogen under both low and high temperatures deteriorated the least of all the storage atmospheres.
4. Low temperature storage of seed in an atmosphere of nitrogen retained better germination percent and germination rate an any of the other storage atmospheres during a 50 week period, but would be operationally difficult to achieve for an improvement in germination of 5.5%.

CHAPTER 4

GROWTH EXPERIMENTS

4.1 INTRODUCTION

A germination index is frequently used as a criterion to assess the worth of a seed sample. However, seedlots found to have similar high levels of germination can show considerable differences in their emergence in the field. Some lots may emerge poorly despite having a high laboratory germination. These lots have come to be described as low vigour seed (Powell and Matthews 1981).

Heydecker (1969) has described what constitutes 'vigour' in the following terms:

> 'All seeds within each seedlot, which is a nominally uniform consignment of seeds, should keep well (should not 'age' rapidly) when stored; when sown, they should germinate simultaneously and without delay; they should be free from seed-borne diseases, and neither the seeds nor the resulting seedlings should be susceptible to microbial interference. The seedlings should be mechanically strong enough to penetrate soil that is compact through cohesion when wet or covered by a hard crust when dry, and they should be capable of establishing themselves despite a wide range of environmental conditions such as extremes of drought and wetness, of cold and heat. While still growing on its own reserves, a seedling should be capable of drawing on these rapidly and of building up whatever metabolites and

tissues are required for the plant to reach the autotrophic state. The growth rate (increase in dry weight) of the young photo-synthesising plants should be high, and each should be capable of rapidly filling the area allocated to it and of producing a high yield of the desired plant part, preferably in short space of time'.

It is clear that vigour is a multicomponent concept rather than a single quantifiable property. However, the International Seed Testing Association has defined seed vigour as the sum total of those properties of the seed which determines the potential level of activity and performance of the seed or seedlot during germination and seedling emergence. The vigour of the seed could express the following properties of the seeds when germinating:

(a) biochemical processes and reactions during germination, such as enzyme reactions and respiratory activity;

(b) rate and uniformity of seed germination and seedling growth;

(c) rate and uniformity of seedling emergence and growth in the field;

(d) emergence ability of seedlings under unfavourable environmental conditions.

In addition to those properties, the effect of seed vigour may persist to influence mature plant growth, crop uniformity and yield (Perry 1980).

Many factors are known to cause variation in seed vigour. One such factor is genotype of the seed which determines the maximum possible vigour. However, it may be modified by environment during maturation on the mother plant, during harvest, and during handling and storage, known to act either separately or together resulting in seed immaturity, variation in size, mechanical damage, deterioration in storage, or invasion by seed-borne fungi. Most of the factors which cause loss of vigour will eventually show up also as a reduction in germination.

From a practical viewpoint, the importance of seed vigour lies in its application in the field. Declining vigour ultimately leads to low germination and a lowered ability to withstand unfavourable conditions (Belcher 1981). Vigorous seeds are at less risk in their germination and establishment in the nursery which, otherwise, may be adversely affected by unfavourable conditions of soil such as compaction, nutrient status, moisture regime, and other environmental conditions. High-vigour seeds may be expected to germinate more synchronously than low-vigour seed, and have high emergence rate and improved seedling growth which can increase the yield and lower production time in the nursery.

As storage condition is one factor which affects seed vigour, samples of the same seed type with similar levels of germination but stored under different conditions may produce seedlings dissimilar in performance depending upon the rate of deterioration in storage (Harrison 1966). The storage atmosphere or

the gas surrounding the seeds is considered one of the storage conditions which may affect the rate of deterioration and hence the vigour of the seed.

A few studies have investigated the effect of different atmospheric gases on the rate of deterioration of agricultural crop seeds during storage. Little attention has been paid to tree seeds and no such studies have been made on *P. radiata* seeds. Therefore, this study was undertaken to investigate the effect of storage atmosphere on the rate of deterioration of seed vigour in *P. radiata*.

Since seed vigour is taken as a qualitative concept rather than a specific property of a seed or seedlot, the level of vigour can be measured only if a specific attribute of the seed is considered (Perry 1980). Seedling growth is one of the attributes of the seed. Measurement of seedling growth is one of the simplest tests for vigour (Perry 1976). It can be objectively evaluated by a continuous variable such as seedling height (Pollock and Roos 1972) but evaluation by dry weight of the seedling (e.g. Harrison 1966) provides a more accurate assessment. For the purpose of this study, dry weight of seedling was used to assess the vigour of the seed which in turn is the measure of the value of storage condition.

This chapter presents and discusses the results obtained from 80 replicated growth tests on samples stored in different atmospheres (air, vacuum, carbon dioxide and nitrogen) under a range of temperatures for a period of 50 weeks.

4.2 MATERIALS AND METHOD

4.2.1 Materials

Seed: Clean and pure seeds of *P. radiata* from the same seedlot as was used in the germination experiments in Chapter 3 were used.

Storage Container: Laminated plastic bags were again used for storage treatment of the seed sample in exactly the same way as described in the previous chapter.

4.2.2 Method

Preparation of samples: Sample reduction was by successive halving of the seedlot in a Gamet divider as already described in the previous chapter. A sample of 200 seeds was used. One hundred samples of 200 seeds were prepared to provide material for 4 storage atmospheres x 5 temperatures x 5 test periods.

Moisture Content of seed: The moisture content of the seedlot had been determined for the germination experiments and was found to be 8.05%.

Storage treatment of samples: The storage treatments were exactly the same as in the germination experiments in Chapter 3. That is:

Atmosphere (a) air
(b) vacuum
(c) carbon dioxide
(d) nitrogen

Temperature (a) 5°C

(b) 15°C

(c) 25°C

(d) 35°C

(e) 45°C

A set of samples, one of air, vacuum, carbon dioxide and nitrogen, was removed from storage every 10 weeks for 50 weeks.

The following elements were involved:

Storage atmosphere: 4

Storage temperature: 5

Storage time: 6 including time 0.

After only 10 weeks, it was evident that seed stored at 45°C had poor seedling emergence irrespective of storage atmosphere and this treatment was subsequently abandoned and was not included in the final analysis. The final analysis is then concerned with 4 x 4 x 6 experimental combinations.

Growth test: The first growth test was carried out for all the storage treatments 10 weeks after the commencement of the experiment. The growth tests were repeated every 10 weeks up to 50 weeks. Each test was replicated 10 times by dividing at random the 200-seed sample into 10 lots of 20 seeds.

Plastic pots, 9 cm diameter and 15 cm deep, were filled with a mixture of vermiculite and perlite in proportion of 1:1. Pots were watered thoroughly and the stored samples of 200 seeds sown at a rate of 20 seeds per pot. Prior to sowing, the seeds were surface sterilised with 1% sodium hypochlorite solution in vacuum for 5 minutes and washed with sterile water. The seeds were sown at a depth of 1 cm and evenly spaced. Pots were labelled and placed in a controlled environment growth cabinet of LB type (Morse and Evans 1962). The pots were placed upon saucers, each 13 cm in diameter and 1 cm deep for watering.

The temperature set at growth cabinet was 19° - 24°C diurnally alternating, each 12 hours. Photoperiod was of 12 hours corresponding to a day temperature of 24°C. The light in the growth cabinet was provided by 80-watt white fluorescent tubes and the intensity at the surface of the pots was 180 microeinstein. The pots were watered every alternate day by filling the saucer which ensured the amount of water given to be equal in all the pots. Liquid complete fertiliser 'Liquifert' was applied to the seedlings at the rate of one teaspoonful in every 5 litres of water. The first application of fertiliser solution was 25 days after sowing and thereafter every 7 days. The test period was 49 days.

At the termination of the test period, all the seedlings in the pots were removed and the roots washed clean of vermiculite and perlite. Seedlings in each pot were counted, placed in separate, labelled paper bags and oven-dried at 85°C for 48 hours. After

cooling in a desiccator for 30 minutes the bulked seedlings in each bag were weighed.

In summary, the growth test involved following elements:

Container:	9cm diameter x 15 cm deep plastic pot.
Number of seed per container:	20.
Number of replications:	10.
Sterilisation:	1% sodium hypochlorite solution in vacuum for 5 minutes.
Germination medium:	1:1 mixture of perlite and vermiculite.
Light:	180 microeinstein intensity provided by fluorescent tubes, 12 hours a day.
Temperature:	19°-24°C diurnally alternating each 12 hours.
Watering:	Every alternate day.
Fertilisation:	First on day 25, thereafter every 7 days.
Test period:	49 days.

4.3 DATA EXPRESSION AND ANALYSIS

The average dry weight of a seedling in a replication was calculated by dividing the total or bulked dry weight of the seedlings germinated by number of the seedlings in that replication. Therefore, the measurements were numerically independent of the germination capacity of the seed sample.

The mean of the average dry weight of each of the 10 replications was taken as the mean dry weight for each treatment. Mean dry weight was plotted against storage time period for each storage temperature and for each storage atmosphere to show graphically the course of deterioration in seed vigour, as measured by seedling dry weight, during the 50 weeks of storage.

For statistical analysis, using analysis of variance, the average dry weight of each of the 10 replications for each treatment was used. Analysis of variance was carried out for the following factors and their interactions:

Factor 1: Atmosphere at 4 levels - air, vacuum, carbon dioxide and nitrogen

Factor 2: Temperature at 4 levels - 5, 15, 25 and 35°C

Factor 3: Time at 6 levels - 10, 20, 30, 40, 50 weeks including a control (0 week)

The GENSTAT (General Statistical) computer programme (1977) was used for the analysis. Differences between treatment means were

also compared by the method of least significant difference (LSD) test (Steel and Torrie 1960). The'F' and 't' distributions were taken from tables of Fisher and Yates (1949).

4.4 RESULTS

The results of all growth experiments are presented in Table 4.1 and show that there were marked differences in seedling mean dry weight among different storage conditions. Analysis of variance of the results (Table 4.2) show that all the main factors gave a significant variance ratio at the .01 probability level. The variance ratio contributed by the time factor (341.63) was the highest, as it was for the rate of germination, followed by atmosphere (250.13) and temperature (50.91) indicating the considerable influence of time in the maintenance of vigour of seed. The interaction between atmosphere and temperature was found to be significant at the .05 probability level and the rest of the first order interactions were significant at the .01 probability level. However, the variance ratios of these interactions were modest compared to those of the main factors. The second order interaction was not significant (P = .05).

From Table 4.1 it can be seen that nitrogen storage of the seeds proved once again to be the best storage treatment. The dry weight of the resulting seedlings was highest when the seeds were stored in nitrogen, followed closely by carbon dioxide, vacuum and

Table 4.1 Mean dry weight (mg) of a seedling pertaining to each growth test for all storage conditions

Storage condition		Storage time in weeks					
Atmosphere	Temperature °C	0	10	20	30	40	50
Air	5	50.0	40.2	51.4	41.4	37.9	35.1
	15	50.0	43.9	49.6	39.6	36.5	34.2
	25	50.0	42.7	54.3	37.1	34.4	33.0
	35	50.0	40.1	51.9	34.1	27.5	24.0
Vacuum	5	50.0	43.5	47.1	42.7	45.1	38.2
	15	50.0	45.1	46.9	41.8	41.8	38.7
	25	50.0	46.9	44.8	40.0	36.8	34.8
	35	50.0	40.2	43.3	38.0	30.7	27.5
Carbon dioxide	5	50.0	57.4	51.5	50.9	46.0	40.5
	15	50.0	61.8	51.1	49.3	45.9	41.2
	25	50.0	59.7	49.7	44.7	45.4	40.4
	35	50.0	58.9	50.2	43.2	41.6	34.0
Nitrogen	5	50.0	58.3	52.1	52.9	44.6	42.3
	15	50.0	60.2	52.6	52.4	46.1	42.2
	25	50.0	61.2	51.4	49.7	44.9	40.8
	35	50.0	61.6	51.0	47.9	41.5	35.5

Table 4.2 Summarised analysis of variance of mean dry weight of seedling presented in Table 4.1

Source of variation	Degrees of freedom	Mean squares	Variance ratio
Atmosphere	3	4.33323	250.13**
Temperature	3	0.88199	50.91**
Time	5	5.91831	341.63**
Atmosphere x Temperature	9	0.03839	2.21*
Atmosphere x Time	15	0.58889	33.99**
Temperature x Time	15	0.15398	8.88**
Atmosphere x Temperature x Time	45	0.01697	0.97NS
Residual	845	0.01732	

** = Significant at P = .01

* = Significant at P = .05

NS = Not significant at P = .05

air, in that order. The means for storage atmospheres were significantly different from one another at the .05 probability level (Table 4.3, top block). It can also be observed that the difference between the mean value of nitrogen (49.5 mg) and carbon dioxide (48.4 mg) was very small and was similar to that between vacuum (42.2 mg) and air (41.2 mg).

Some variations in the results at early test periods were observed. This may have been due to:

(a) damping-off problem, and

(b) high temperature fluctuation in one of the growth cabinet leading to breaking down of the growth cabinet in the middle of the experiment. This required a switch over temporarily to another type of the growth cabinet. Both of the problems were overcome later.

At 5°C and 15°C, seed stored in nitrogen and carbon dioxide was able to maintain for a longer time period the original high value of seedling dry weight compared to vacuum and air storage. This is shown by the markedly different mean dry weights between the treatments at the end of the 50 week storage period. When stored at 35°C, the deterioration in dry weight of the seedling was substantial among all four storage atmospheres even at 40 weeks storage (Plate 4.1 shows differences in growth between seedlings from air, carbon dioxide and nitrogen). Seeds stored in air gave the lowest mean dry

Table 4.3 General mean dry weight (mg) of a seedling for different storage atmospheres, temperatures and time periods.

Mean values which do not have the same affix are significantly different within a block at .05 probability level according to LSD test.

Storage atmosphere			
Air	Vacuum	Carbon dioxide	Nitrogen
41.2^{a}	42.2^{b}	48.4^{c}	49.5^{d}

Storage temperature (°C)			
5	15	25	35
46.6^{a}	46.7^{a}	45.5^{b}	42.6^{c}

Storage time (weeks)					
0	10	20	30	40	50
50.0^{a}	51.3^{b}	49.1^{c}	44.1^{d}	40.4^{e}	36.4^{f}

Standard error of difference between means for

(a) atmosphere and temperature = 0.3

(b) storage time = 0.4

Least significant difference value for

(a) atmosphere and temperature = 0.6

(b) storage time = 0.8

Plate 4.1 Comparative growth of seedlings at day 25, from seeds stored at different temperatures in air (A), carbon dioxide (B) and nitrogen (C) for 40 weeks.

A

35°C 25°C 15°C 5°C

B

35°C 25°C 15°C 5°C

(Continued)

C

35°C 25°C 15°C 5°C

weight of a seedling (24.0 mg) compared to the highest value of 35.1 mg for nitrogen storage at 50 weeks storage period (Plate 4.2). Put another way, the mean dry weight of a seedling raised from seed stored in nitrogen at 35°C for 50 weeks was 46% heavier than a seedling raised from seed stored in air at a similar temperature and for the same period.

Statistical analysis of the means of storage temperatures show that there was no significant difference between 5°C and 15°C at the .05 probability level (Table 4.3, middle block). However, means for the other temperatures of 25°C and 35°C showed significant differences between themselves and the other temperatures (P = .05).

Table 4.3, last block, gives a statistical comparison of mean dry weight of a seedling for each seed storage time period. The means for all storage periods, including week 0, were significantly different from one another at the .05 probability level.

Figure 4.1 presents graphically the comparative course of mean dry weight of a seedling for each seed storage atmosphere under different temperature conditions over a period of 50 weeks, confirming the superiority of nitrogen storage of seed over other three storage atmospheres in maintainning the vigour of the resulting seedlings.

A

5°C 15°C 25°C 35°C

B

5°C 15°C 25°C 35°C

Plate 4.2 Comparative growth of seedlings at day 25, from seeds stored at different temperatures in air (A) and nitrogen (B) for 50 weeks.

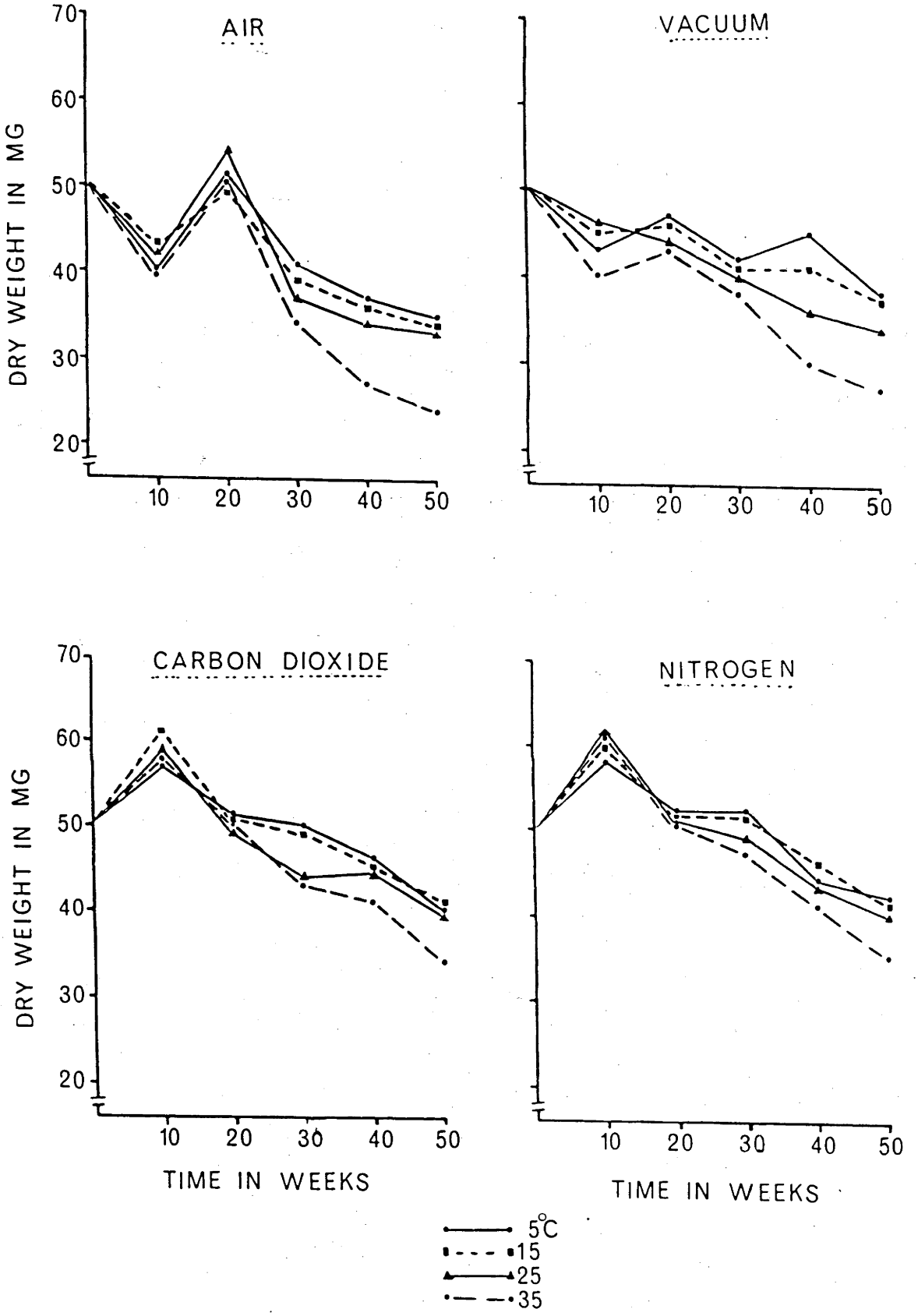

Figure 4.1 Comparative course of deterioration in mean dry weight of a seedling for each seed storage temperature for each storage atmosphere for 50 weeks.

4.5 DISCUSSION

Statistical analysis of the results has shown that the time factor contributed the highest variance ratio as with rate of germination. This indicates over a relatively short period of 50 weeks that seed deterioration is manifested more in loss of vigour than by loss of viability. The results obtained in this experiment showed that there were marked differences in mean dry weight of the seedling among different storage conditions. These differences must have been due to differences in the deterioration rate of the seed brought about by the different storage conditions, affecting subsequent seedling growth accordingly. Further, the results have shown that the mean dry weight of the seedling was highest for seed stored in an atmosphere of nitrogen and progressively lower in carbon dioxide, vacuum and lowest in the case of air sealed storage. Seedlings from nitrogen, carbon dioxide and vacuum storage were 19.5%, 17.1% and 2.4% heavier than those from air storage respectively. Similar results were obtained for rate of germination in the previous chapter. Harrison (1966) also found better results with nitrogen storage in the case of lettuce seeds. He found that lettuce seeds, 6% moisture content stored at 18°C for 3 years, gave a mean weight of a seedling of 5.10 g in sealed air, 6.80 g in vacuum and carbon dioxide, and 7.09 g in nitrogen as compared to 4.54 g in open air and 1.3 g in pure oxygen. He observed similar results with other species, such as onion. This led him to note that the ability of nitrogen to delay or slow down deterioration more than other atmospehric gases appears to be more than a chance effect. In the

present study the rate of deterioration of seed in sealed air is higher than vacuum, carbon dioxide and nitrogen because of the deleterious effect of oxygen. Though the presence of oxygen has a major influence on seed deterioration, the differences in rate of seed determination among oxygen-free atmospheres of nitrogen, carbon dioxide and vacuum suggests that there is also some other factors involved.

Even at lower temperatures of 5°C and 15°C, seeds stored in all the four atmospheres did not maintain their original vigour though at these temperatures much of the original high level of viabilty was maintained. The decrease in seed vigour in air sealed was quite marked but progressively less in vacuum, carbon dioxide and nitrogen as compared to control (0 weeks). At the end of 50 weeks storage the decline in seedling dry weight from seeds sealed in air was 30% while it was 16% in nitrogen and 18% in carbon dioxide as compared to control. The substantially greater loss in dry weight in air sealed compared to the carbon dioxide and nitrogen atmospheres shows quite clearly the deleterious effect of oxygen on seed vigour even at lower temperature. Similar marked differences were observed between air sealed and nitrogen storage for rate of germination. In other words, there was a decrease in seed vigour before a loss of viability was evident. This observation is in accord with similar findings by Woodstock (1969) and Roberts (1979).

The results showed also that at temperatures as high as 35°C the loss in seed vigour of seed stored in nitrogen or carbon dioxide was comparatively less than that of air sealed seed.

Although these results support the hypothesis that oxygen is deleterious to the maintenance of seed vigour, the mechanism by which it affects the seed has not yet been ascertained.

Though the differences in seed vigour among different atmospheres were marked, these differences can be expected to be exaggerated when seedlings are grown under conditions of greater stress in the field. Heydecker (1960) noted that germination under conditions of stress, such as lack of moisture, reveal greater differences in vigour than are evident from germination testing under optimal conditions.

It can be concluded that irrespective of the storage temperatures, whether low or high, the presence of oxygen is detrimental to the maintenance of seed vigour in *P. radiata*. The vigour of *P. radiata* seeds is maintained better in an oxygen-free atmosphere and at low temperatures. Storage in an atmosphere of nitrogen is marginally better than in carbon dioxide and considerably better than in a vacuum for maintaining seed vigour.

4.6 SUMMARY OF RESULTS

The second aim of this study was to evaluate the effects of sealed storage atmospheres of air, vacuum, carbon dioxide and nitrogen gas on seed vigour (as measured by seedling growth) after a seed storage period of up to 1 year. The results of the experimental work are summarised as follows:

1. Statistical analysis of the results showed significant variance ratios (F value) for all main factors - storage atmosphere, temperature and time - at the 1% level of probability.
2. The dry weight of the seedling, an index of seed vigour, was highest when the seed was stored in an atmosphere of nitrogen followed by carbon dioxide, vacuum and air, in that order.
3. Low temperature storage of seed in an atmosphere of nitrogen was superior to any other form of storage in maintaining seed vigour.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

The amount of tree seed used for afforestation and reforestation is increasing rapidly as projects to provide industrial wood (mainly saw logs, pulpwood and pitprops), non-industrial wood (fuelwood), and protection forests accelerate. The majority of these new plantations are in developing countries in the tropics (Lanly 1982, Montalembert and Clement 1983).

The provision of seed of good quality is of fundamental importance in increasing nursery efficiency and plantation productivity (Turnbull 1983). Upgrading seed quality factors, such as germination percentage, seed vigour and purity has been hindered in many developing countries by lack of appropriate technical methods and facilities. The processing, testing and storage of seeds is often poorly developed and carried out inefficiently with methods that have been inadequately researched. An analysis of seed problems in developing countries of Asia, Africa and Latin America by Kamra (1974) concluded "the greatest difficulties lie in the fields of production, processing, testing, storage and certification of seed. The facilities for these purposes are either absent or unsatisfactory. The situation is even made worse by the fact that there is a great lack of equipment and trained personnel in this field".

Proper storage of forest tree seed is of practical importance in providing good quality seed whenever it is required. However, in developing countries there are many problems related to implementing good storage practices. One problem is the absence or inadequacy of storage facilities in areas where species exhibit periodicity in their seed crops.

Periodicity of seeding of some species has made it difficult in many developing countries to collect an adequate quantity of seed each year to maintain a plantation programme. *Pinus roxburghii* has been one of the main plantation species in Nepal and India for the production of building material, resin and pulp wood (Shrestha 1975, Ghosh and Singh 1973) but it fails to produce seed every year (Troup 1921). Similar problems exist for *Pinus wallichiana* and *Cedrus deodora*, and it is especially acute in the case of a poor seeder such as *Abies pindrow* in which good seed years are spasmodic, often many years apart. Species of bamboo, such as *Dendrocalamus* and *Bambusa* species, are extensively planted in India and other parts of South East Asia and serve as an important source of long-fibre raw material for paper and pulp manufacture and have many other uses. These species seed very rarely, possibly only once in 20-40 years (Ghosh and Singh 1973). In the absence of, or inadequacy of proper storage facilities and techniques, the advantage cannot be taken of a good seed year of these species. In countries, such as Nepal, seeds are stored in tins and polythene bags at ambient room conditions with the consequence that there is rapid seed deterioration and when sown in the nursery, the seeds give poor germination and produce seedlings of inferior quality.

Even when cold storage is available optimum storage methods have not always been determined. For example, *Cedrela odorata* is a valuable species in West African countries, such as Ghana and Nigeria but seedlots completely lose their viability within a few months at 25°C (Brookman-Amissah 1973) and even at 2-4°C the viability is retained for only a short period (Lamb 1968).

The problem of seed storage is aggravated further in the developing countries having a tropical climate. In such countries, the ambient high humidity and temperature results in a rapid loss of seed viability during storage in the absence of proper storage facilities. Forests seeds are often treated in the same way as agricultural crop seeds which may be dried in the sun and stored only in ambient rooms in seed containers such as paper, cloth, polythene bags, tin cans and drums (Hor 1977). Although seeds dried to a lower moisture content can retain their viability for a relatively longer period, where there is a high level of humidity the moisture content of seeds stored in non-moisture proof containers will gradually increase to equilibrium moisture content. In addition to physiological deterioration at high temperatures and humidities, seed pests and diseases are favoured.

The adversity of the tropical moist ambient environment can be overcome by lowering the temperature and relative humidity of the storage atmosphere and lowering the seed moisture content. Under these conditions, seed viability can be maintained for a longer period but refrigeration and dehumidification equipment is required. However, these installations are sophisticated, costly

and difficult to maintain, and beyond the reach of most developing countries. In these countries, where funds are scarce and there is a shortage of trained personnel, what is needed is a simple, cheap and reasonably foolproof storage that will safely maintain seed viability and vigour against the dangers of high temperature, high moisture, insects and diseases (Harrington and Douglas 1970). For example, in Nepal, seed storage methods which rely heavily on the control of storage temperature by refrigeration and the maintenance of low seed moisture by dehumidification equipment are not appropriate because of the unreliability of the electricity supply and the unavailability of trained personnel for maintenance of the equipment. Even the provision of a central seed storage facility in a major city may not solve the seed storage problem in some developing countries because of a lack of efficient communications. The difficulty of supplying seeds to field areas from a central storage facility is not practical when nurseries and plantations are being established in remote areas, as in community forestry projects at the village level in Nepal (Shepherd 1981). What is needed in such situations is a simple and easy storage method for short or medium term periods at a location close to the nursery site.

It is clear that the problems of seed storage in developing countries centres either on the lack or inadequacy of the storage facilities or on the absence of suitable storage techniques. Simple and cheap methods which have proved successful in maintaining viability and vigour of the seed can be employed to improve seed storage conditions without resorting to more complex, sophisticated

storage equipment. In the present study of controlled atmosphere storage, reduction in the rate of deterioration of seed viability and vigour is achieved by replacing or limiting the oxygen supply in a sealed container. The use of sealed laminated plastic bags with proven impermeability to moisture and gases effectively eliminates the need to use a dehumidified room to control seed moisture content.

The present study showed that the viability and vigour of *P. radiata* seeds can be prolonged by controlling the atmosphere in which the seed is stored. Storage in air is less effective than in an atmosphere of nitrogen, carbon dioxide, or in a vacuum. Of these alternatives nitrogen was the most effective in maintaining viability and vigour of the radiata pine seeds at all the storage temperatures employed. Although these results relate directly to *P. radiata*, they should be applicable to orthodox seeds of other plantations species used in the tropics and subtropics.

The choice of a particular storage method will depend largely on the length of time the seed is to be stored and the cost involved. For long term storage there would be an advantage in using a nitrogen atmosphere but where medium term storage is sufficient carbon dioxide could be employed. Stored in carbon dioxide, seeds maintain comparable viability and vigour level, and the gas is cheaper and generally more readily available than nitrogen. A distinctive advantage is that carbon dioxide has a sterilisation effect on the growth of fungi and other micro-organisms (Mitsuda and Yamamoto 1980) and on insects (Bailey and Banks 1980). A further benefit in storing seed in carbon dioxide is the

saving in storage space and ease of transportation due to the adsorption phenomenon. The storage bags can be packed more easily because they shrink onto the seed in much the same manner as with vacuum storage.

The deterioration of seed during transportation from one region to another or from one country to another, has also been a problem frequently encountered in tropical climates. Seeds packaged in sealed laminated polythene bags with carbon dioxide or nitrogen will not adsorb moisture and will be less affected by high temperatures. Therefore, the storage methods investigated in the present study will be beneficial if applied to the transportation of seeds, especially in humid tropical climates. These storage methods could be applicable particularly for use in central seed stores, where bulk storage would be anticipated and there would be a ready availability of nitrogen and carbon dioxide for packaging smaller lots for transport. In the field areas, where seed storage is mostly short term, and gases less available, storage of low moisture content seed in sealed containers in air or in a vacuum would be simple, cheap and viable methods.

The maintenance of a high level of seed vigour during storage has practical importance. Seedling establishment in the field is generally competitive and a number of factors, such as rapidity of germination and rate of growth of the seedlings, may determine which seedlings survive to maturity. Vigorously growing seedlings are also more resistant to soil pathogens, one of the factors responsible for seedling mortality in the field, and they

will have a better chance of surviving stress conditions such as drought. Therefore, the storage method employed should maintain not only a high level of viability but also a higher level of seed quality in the nursery.

Seed storage is a very important stage in the handling of seed from the time of collection to when it is sown in the nursery. The maintenance of seed quality during the storage period is crucial to subsequent seed performance so that the application of simple but effective techniques will have considerable value in enhancing the results of forestry plantations in developing countries. The use of controlled atmosphere storage in impermeable laminated polythene bags as has been investigated in this study, is such a technique.

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